U.S. National Institute of Health.

RA
421
U4
no.27
cop.2
Biological
& Medical
Serials

TREASURY DEPARTMENT.

Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 27.

M. J. ROSENAU, Director.

APRIL 9, 1906.

Biological
& Medical
Serials

THE LIMITATIONS OF FORMALDEHYDE GAS AS A DISINFECTANT,

WITH SPECIAL REFERENCE TO CAR SANITATION.

BY

THOMAS B. McCLINTIC.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1906.

NOTICE TO LIBRARIANS AND BIBLIOGRAPHERS CONCERNING THE SERIAL PUBLICATIONS OF THIS LABORATORY.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

The following *bulletins* (Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.) have been issued:

No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition March, 1904.)

No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

No. 18—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

(Continued on third page of cover.)

TREASURY DEPARTMENT.

Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 27.

M. J. ROSENAU, Director.

APRIL 9, 1906.

THE LIMITATIONS OF FORMALDEHYDE GAS AS A DISINFECTANT,

WITH SPECIAL REFERENCE TO CAR SANITATION.

BY

THOMAS B. McCLINTIC.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1906.

ORGANIZATION OF HYGIENIC LABORATORY.

WALTER WYMAN, *Surgeon-General,*
United States Public Health and Marine-Hospital Service.

ADVISORY BOARD.

Maj. Walter D. McCaw, Surgeon, U. S. Army; Surgeon John F. Urie, U. S. Navy; Dr. A. D. Melvin, Chief of U. S. Bureau of Animal Industry, and Milton J. Rosenau, U. S. Public Health and Marine-Hospital Service, *ex officio*.

Prof. William H. Welch, Johns Hopkins University, Baltimore, Md.; Prof. Simon Flexner, Rockefeller Institute for Medical Research, New York; Prof. Victor C. Vaughan, University of Michigan, Ann Arbor, Mich.; Prof. William T. Sedgwick, Massachusetts Institute of Technology, Boston, Mass., and Prof. Frank F. Wesbrook, University of Minnesota, Minneapolis, Minn.

LABORATORY CORPS.

Director.—Passed Assistant Surgeon Milton J. Rosenau.

Assistant director.—Passed Assistant Surgeon John F. Anderson.

Pharmacist.—Frank J. Herty, Ph. G.

Acting librarian.—E. B. K. Foltz.

DIVISION OF PATHOLOGY AND BACTERIOLOGY.

Chief of division.—Passed Assistant Surgeon Milton J. Rosenau.

Assistants.—Passed Assistant Surgeons John F. Anderson, W. W. King, H. A. Stansfield, and T. B. McClintic.

DIVISION OF ZOOLOGY.

Chief of division.—Ch. Wardell Stiles, Ph. D.

Assistants.—Passed Assistant Surgeon Joseph Goldberger, Philip E. Garrison, M. D., and Arthur L. Murray, M. D.

DIVISION OF PHARMACOLOGY.

Chief of division.—Reid Hunt, Ph. D., M. D.

Assistant.—Madison B. Porch, B. S.

DIVISION OF CHEMISTRY.

Chief of division.—Joseph H. Kastle, Ph. D.

Assistant.—H. L. Amoss, B. S.

CONTENTS.

Page.

Introduction 7

Experiments in a special room 9

  Description of room 9

  Determination of the amount of formaldehyde in the atmosphere of the room . . 10

    Potassium cyanide solution 10

      (*a*) Absorption in cyanide 13

      (*b*) Absorption in water 14

  Temperature and humidity 15

  Bacteriological methods 16

  Disinfectants authorized by U. S. Quarantine Regulations and the proper methods of generating and using same 18

  The different methods of evolving formaldehyde gas 20

    Formalin-permanganate 20

    Diluted formalin-permanganate 30

    Retort without pressure 40

    Autoclave 46

    Sheet-spraying 52

    Generating lamp 56

    Formalin-aluminum sulphate-lime 62

Experiments in a large bottle 66

  Formalin-permanganate method 66

    Percentage of formaldehyde liberated 66

    Percentage of formaldehyde remaining in the residue 67

  Diluted formalin—permanganate method 69

  Drying formaldehyde gas by anhydrous calcium chloride 69

  Humidity and temperature in formaldehyde disinfection 71

Disinfection of sleeping cars with formaldehyde gas 77

  Formalin-permanganate method 78

  Diluted formalin-permanganate method 96

  Retort without pressure 99

  Autoclave 102

  Generating lamp 105

Summary and conclusions 109

THE LIMITATIONS OF FORMALDEHYDE GAS AS A DISINFECTANT, WITH SPECIAL REFERENCE TO CAR SANITATION.

By Thomas B. McClintic,

Passed Assistant Surgeon, U. S. Public Health and Marine-Hospital Service.[a]

INTRODUCTION.

This work was undertaken in order to throw further light upon the much discussed question as to the merits of formaldehyde gas as a germicidal agent and the best method of obtaining it for disinfecting purposes, a problem which has been of much interest to the Service in its public health work, particularly in its relation to car sanitation.

Some preliminary experiments soon showed certain limitations to the use of formaldehyde gas as a disinfectant, and on account of the practical importance of these limitiations they were taken up and studied under definite conditions.

A special study was made of the production of formaldehyde gas by the action of potassium permanganate on formalin. When these two substances are brought together a vigorous reaction takes place accompanied by rapid liberation of formaldehyde gas.

Although this is a comparatively new method of liberating the gas it is not clear, from a review of the literature, who was the first to suggest it. Certainly among the first to describe it was Dr. G. F. Johnson, of Sioux City, Iowa; this was done in a paper read at the eighth semiannual meeting of the Sioux Valley Medical Association held at Sioux City, Iowa, January 21 and 22, 1904. In the spring and summer of the same year Henry D. Evans,[b] chemist, and Dr. J. P. Russell, bacteriologist, of the Laboratory of Hygiene, Augusta, Me., conducted experiments with formaldehyde gas liberated by this method;

---

[a] Acknowledgments: To Daniel Base, Ph. D., technical assistant in pharmacology, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service, I am indebted for the chemical determinations made during warm weather and for assistance in the preparation of the corresponding part of the text of this bulletin; and to Madison B. Porch, B. S., assistant in the division of pharmacology, Hygienic Laboratory, for assistance in making the chemical determinations during cold weather.

[b] Evans, Henry D., and J. P. Russell: Formaldehyde disinfection. 13th Ann. Rep. Maine State Board of Health.

they made determinations of the percentage of gas liberated and tests of its properties as a disinfectant. Their results indicated this method of liberating the gas to be an efficient as well as an ideal one as regards simplicity and rapidity.

As the published reports of almost innumerable experiments with formaldehyde give such contradictory results, a further study of this question by us has resulted not only in explaining the reason of these contradictory results, but also has developed distinct limitations to the use of the gas as a disinfecting agent.

For comparison with the formalin-permanganate method many experiments under similar conditions were also carried out with each of the other well-known methods of obtaining formaldehyde gas, namely, the retort, autoclave, generating lamp, sheet-spraying, and formalin-aluminum sulphate-lime methods.

In order to make our comparisons complete, it was necessary to know, in addition to the relative power for destroying microorganic life, the quantity of formaldehyde gas present in the atmosphere of the room; or, in other words, the yield of gas from a definite amount of formalin by the various methods employed. A room was chosen which afforded special advantages for conducting chemical as well as bacteriological experiments. Quantitative chemical determinations of the percentage of gas liberated were made simultaneously with bactericidal tests by drawing a definite volume of air from the room through an absorbing solution and determining the amount of formaldehyde absorbed.

Our method of making the bacteriological exposures in the experiments in the room differs somewhat from those usually employed by other experimenters. The difference consists principally in having a room provided with special apparatus, so that the cultures of the different organisms can be introduced into and removed from the formaldehyde in the room and planted in bouillon whenever desired. This enabled us to ascertain almost exactly the time that a given charge of formaldehyde required to exert its germicidal action upon the different organisms exposed. By making the chemical determinations simultaneously with the bacteriological tests the quantity of formaldehyde per cubic foot of air in the room at certain stages of the experiment was ascertained. The results of a few experiments in this room showed that the formalin-permanganate method, besides being rapid and simple, compared very favorably with the retort and auctoclave methods as regards the percentage of formaldehyde gas liberated and its power to destroy microorganic life.

A request was made of the local management of the Pullman Company for the use of some of their cars for experimental purposes, which was cheerfully granted. The work was taken up in railroad cars and a number of experiments conducted with various methods of liberating the gas.

As our studies progressed it became evident that changes in climatic conditions caused a wide variation in our results. Consequently our work was continued through the different seasons of the year in order to observe the influence of temperatures, humidity, wind, etc.; but as the germicidal power of the gas during cold weather was very unsatisfactory, only a limited number of experiments were necessary during the winter season.

A number of experiments with the formalin-permanganate method were performed in large glass bottles. In this manner it was possible to control or eliminate some of the atmospheric conditions with which we had to contend in the room and cars. Particular attention was given to the influence of humidity upon the bactericidal power of formaldehyde, as well as to the percentage of formaldehyde liberated and that remaining in the residue.

Our bacteriological experiments were carried out with the purpose principally of determining the properties of formaldehyde gas as a surface disinfectant, it being taken for granted that formaldehyde possesses only slight penetrating power.

In the following pages the work is given under three headings, namely—

1. Experiments in a special room.
2. Experiments in a large bottle with the formalin-permanganate method.
3. Disinfection of sleeping cars with formaldehyde gas.

EXPERIMENTS IN A SPECIAL ROOM.

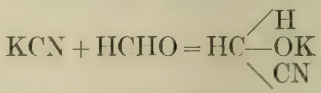
DESCRIPTION OF ROOM.

The room into which the formaldehyde gas was liberated contained 2,000 cubic feet of air space. The walls, floor, and ceiling were of ordinary tongue and groove construction, and in addition to this the room was lined throughout, except over the windows, with sheet zinc, closely nailed at the joints. There were two windows and two doors, all of which were made to fit closely. The windows were on opposite sides and the doors opened into adjoining rooms, so that the experiment room was freely exposed to the winds on two sides. While comparatively close, it was of course not air-tight.

One wall was pierced by three horizontal rows of zinc tubes about 2¼ inches in diameter, one row being about a foot from the floor, one midway between the floor and the ceiling, and one about a foot from the ceiling. Each tube was provided with a sliding wooden frame carrying a small wire tray for the purpose of making the bacteriological exposures. This frame was long enough so that when in position the wire tray would be entirely within the room containing the formaldehyde, the outer end of the frame being pushed just far enough into the tube through which it was introduced to close the outer opening of the tube with a tight-fitting rubber stopper. Some of these zinc tubes were also utilized for drawing air from the room in making the chemical determinations.

DETERMINATION OF THE AMOUNT OF FORMALDEHYDE IN THE ATMOSPHERE OF THE ROOM.

The quantity of formaldehyde in the atmosphere of the room during any particular experiment was determined by the potassium cyanide method of Romijn. This method depends upon the fact that formaldehyde combines with potassium cyanide in the sense of this equation:



$$\mathrm{KCN} + \mathrm{HCHO} = \mathrm{HC}\begin{array}{l}\diagup \mathrm{H}\\ \text{—}\mathrm{OK}\\ \diagdown \mathrm{CN}\end{array}$$

and that the cyanide thus combined can not be precipitated by means of silver nitrate. On the other hand the excess of potassium cyanide, over and above that required by the aldehyde, reacts with silver nitrate in the sense of this equation:

$$\mathrm{KCN} + \mathrm{AgNO_3} = \mathrm{AgCN} + \mathrm{KNO_3},$$

and hence can be determined quantitatively by means of decinormal solutions of silver nitrate and potassium sulphocyanate.

The potassium cyanide solution.—This is made by dissolving 3.3 gm. (of purity of 96 per cent or over) in water to the volume of 500 c. c. The solution is standardized against a decinormal solution of silver nitrate in the following manner:

Into a flask graduated to 100 c. c. an excess of the silver nitrate solution is drawn from a burette, say 12 or 13 c. c.; to this are added 4 or 5 drops of strong nitric acid (about 50 per cent), and then 10 c. c. of the cyanide solution from a burette. The flask is then shaken and filled up to the mark with water and the contents thoroughly mixed. The liquid is then filtered into a dry burette and 50 c. c. of the filtrate drawn into a beaker. To this is added some ferric ammonium alum indicator and the excess silver is titrated with a standard solution of potassium sulphocyanate in the usual way. The sulphocyanate solution must also be titrated against the silver solution in the presence of nitric acid and iron alum, to establish its equivalence to the silver nitrate. The number of c. c. of sulphocyanate required is multiplied by 2 and this is converted into its equivalent of decinormal silver nitrate solution. The latter is the excess of silver solution and the difference between it and the volume of silver solution originally taken

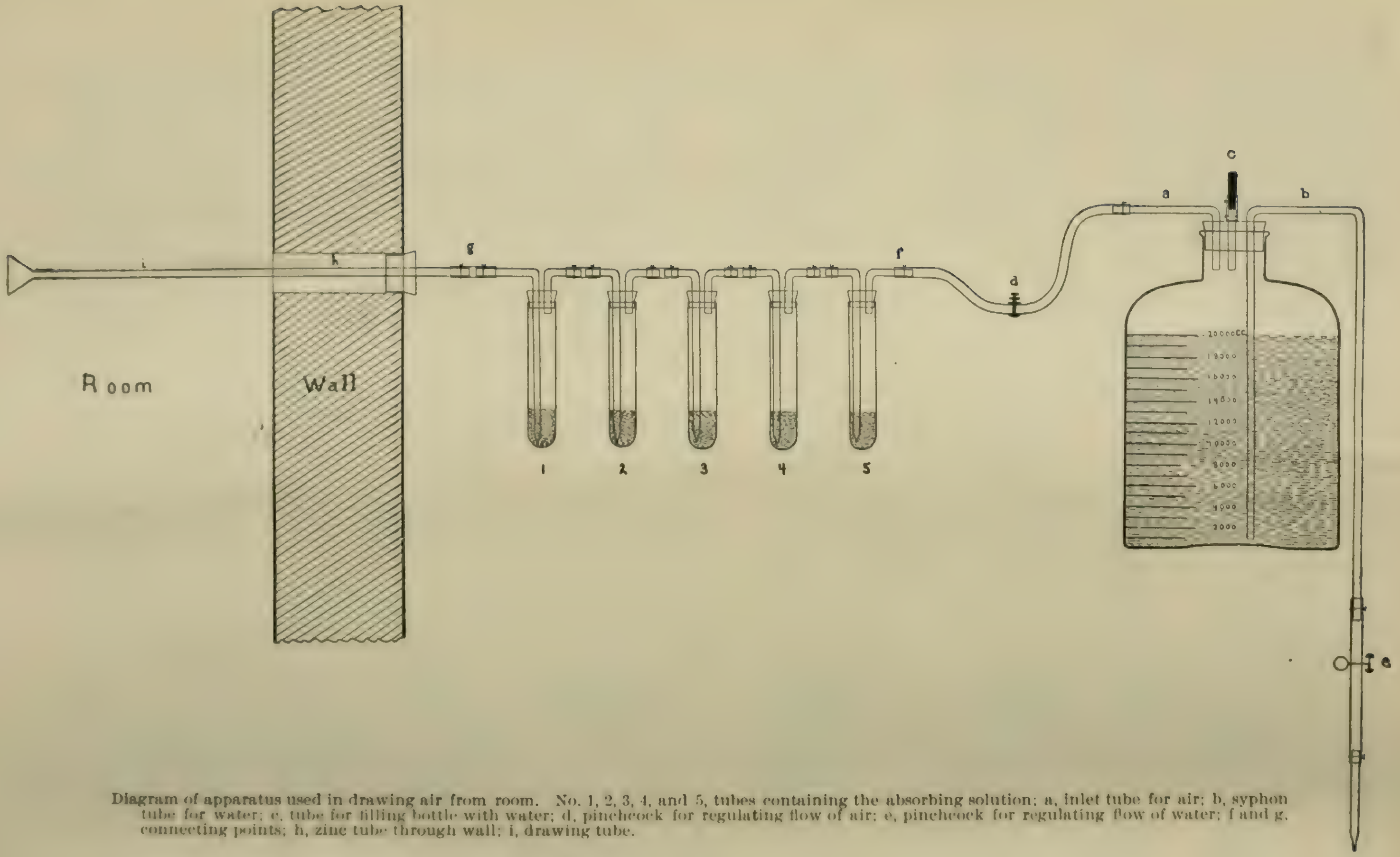


Diagram of apparatus used in drawing air from room. No. 1, 2, 3, 4, and 5, tubes containing the absorbing solution; a, inlet tube for air; b, syphon tube for water; c, tube for filling bottle with water; d, pinchcock for regulating flow of air; e, pinchcock for regulating flow of water; f and g, connecting points; h, zinc tube through wall; i, drawing tube.

gives the number of c. c., to which 10 c. c. of potassium cyanide solution are equivalent.

In the titration of a dilute formaldehyde solution a definite volume or weight (known not to be in excess) is added to 10 c. c., or more if necessary, of the standard potassium cyanide solution. The latter, after stirring, is added to an acidified excess of decinormal silver nitrate solution, the whole made up to 100 c. c. or 200 c. c., and 50 or 100 c. c. of the clear filtrate titrated with sulphocyanate as above. From the amount of sulphocyanate required the volume of silver nitrate precipitated by potassium cyanide is calculated as above, and the difference between this and the volume of silver nitrate equivalent to the amount of cyanide solution originally taken is the number of c. c. of silver solution that represents the formaldehyde present; this number of c. c. multiplied by 0.003 (more exactly 0.002979) gives the weight of absolute formaldehyde, HCHO.

The silver cyanide precipitate must be removed before titrating an excess of silver by sulphocyanate, because it interferes with the sharpness of the end point and causes error.

In order now to determine the quantity of formaldehyde in the atmosphere of the room during any particular experiment a given volume of air, usually 10 liters, was drawn through a series of tubes containing a known amount of the standard solution of potassium cyanide or water by means of the apparatus shown in the cut facing this page. The quantity of formaldehyde absorbed under these conditions was determined in the manner already described. It was soon discovered that the air bubbling through the solution carried away a little hydrocyanic acid. To avoid loss of the latter the last tube containing the cyanide solution was followed by one containing silver nitrate solution, which effectually precipitated all the hydrocyanic acid carried over into it. The silver nitrate tube was followed by one containing distilled water. This tube showed no trace of hydrocyanic acid when tested by the ferric ferrocyanide reaction, and no formaldehyde when tested by Schiff's fuchsin-bisulphite reagent,[a] or ammonia silver nitrate solution.

In preliminary experiments the contents of the silver nitrate tubes, after having passed formaldehyde-laden air through the series, were tested for formaldehyde to determine if any had escaped absorption in the cyanide tubes. To some of the liquid, about 2 c. c. of Schiff's reagent were added; a precipitate of silver chloride was produced, but no pink or purple color, indicating the absence of formaldehyde. For comparison the reagent was also added to a few c. c. of silver nitrate solution to which had been added a trace of formaldehyde; the contents of the tube soon acquired a pink color. It seemed evident that formaldehyde is wholly absorbed from air passing through a series of tubes containing cyanide solution. This conclusion was further strengthened by experiments on the absorption of formaldehyde from air by water alone, which will be mentioned presently.

---

[a] Schiff's reagent: This is prepared by adding 20 c. c. of a solution of sodium bisulphite (sp. gr. 1.27) to 1,000 c. c. of aqueous fuchsin solution (1:1,000) and, after one hour, adding 10 c. c. of pure concentrated hydrochloric acid. It should be preserved in a well-stoppered bottle.

It is a well-known fact that a solution of cyanide undergoes slow oxidation and other changes when exposed to the atmosphere, and it was suspected that in the experiments the long-continued bubbling of air through the solution would have the effect of diminishing its titre. It was found in several trials that when air was passed through 10 c. c. of a nearly decinormal solution of potassium cyanide at the same rate and for the same length of time as in the experiments its equivalent in decinormal silver nitrate was diminished by nearly 0.1 c. c. This correction was applied in the calculation of the result of each determination. Although it may not be absolutely correct, yet it can not be far from correct. As described above, the cyanide tube was followed by a tube containing silver nitrate, which latter was of course used in the subsequent titration.

After nearly all the results given further on had been obtained by the cyanide method, there came to our notice an article by Trillat,[a] in which the author states that formaldehyde can be completely absorbed from air by passing it through a sufficient number of absorption apparatus containing water alone. This suggestion was put to the test, and it was found that three water tubes absorbed all of the formaldehyde, the last tube containing only a trace. The reason for not having chosen water at the start as an absorbing medium was that it was supposed a solution containing a substance, as cyanide, which could combine chemically with formaldehyde would be better suited as an absorbing agent than pure water. Owing to circumstances it was not possible to repeat all of the experiments and use water tubes to absorb the formaldehyde, but in several instances the absorption from the same charged air of the room was carried out in cyanide tubes and in water tubes, side by side. Although three tubes are sufficient, five tubes of water were used for greater precaution. In these parallel experiments the percentage results in the case of absorption by water were from 1.5 to 2 per cent higher than those in the case of absorption by cyanide.

We can not account for this difference, unless it is involved in the uncertainty of the correction made on account of the change of titre of the cyanide solution, due to air bubbling through it, which is referred to on page 12. Without making the correction, the two results were identical in some cases. It is possible also that the greater simplicity in the case of the water tubes causes less error. At all events, although the results tabulated below may be 1.5 to 2 per cent less than the true ones, they are relatively correct. It would require too much space to give the calculation involved in obtaining each result tabulated below, but in order to give an idea of quantities of reagents used and method of carrying out the titrations one experiment will be described in full. It is one in which the air of the room was passed through cyanide solution and through plain water simultaneously.

---

[a] Trillat, A.: Présence normal de la formaldéhyde dans les produits de la combustion incomplete. Rev. d'hyg., vol. 27, no. 2, Feb. 20, 1905.

(*a*) *Absorption in cyanide solution.*—

Tube 1 contained about 8 c. c. KCN solution.
Tube 2 contained about 5 c. c. KCN solution.
Tube 3 contained about 2 c. c. KCN solution.
Tube 4 contained about 8 c. c. $\frac{n}{10}$ $AgNO_3$.
Tube 5 contained about 10 c. c. distilled water.

Only the total quantity of cyanide need be measured, which, in this case, was exactly 15 c. c., distributed about as stated. There should not be less than 8 or 9 c. c. of cyanide solution in the first tube, lest there be an excess of uncombined formaldehyde in the liquid at the end of the absorption. Distilled water was added to the three cyanide tubes to make the column of liquid about 1.5 inches high, and to the silver nitrate tube to make a column about 1 inch high. The tubes were connected and air drawn through them as described above on page 12.

Volume of air drawn from room=10 liters.
Time required to draw air=1 hour, 4 minutes.

The five tubes were disconnected. Seven c. c. of $\frac{n}{10}$ $AgNO_3$ solution were introduced into a 250 c. c. flask and 8 or 10 drops of strong nitric acid (about 50 per cent). Through a funnel the silver-nitrate solution in tube 4 and its connecting tubes were carefully rinsed into the flask, followed in like manner by tubes 1, 2, and 3. Then the flask was filled to the mark with water and the contents thoroughly shaken. One hundred c. c. of the clear filtrate were titrated with sulphocyanate for excess of silver nitrate.

100 c. c. required 1.4 c. c. KCNS solution.
260 c. c. required 3.5 c. c. KCNS solution.
3.5 c. c. KCNS solution×1.021 =
3. 57 c. c. $\frac{n}{10}$ $AgNO_3$ sol. =Excess present.
15.00 c. c. $\frac{n}{10}$ $AgNO_3$ sol. =Quantity taken.
Difference, 11.43 c. c. $\frac{n}{10}$ $AgNO_3$ sol. =Quantity combined with KCN.
15 c. c. KCNS solution × 0.954 =
14. 31 c. c. $\frac{n}{10}$ $AgNO_3$ sol. =Quantity that would have been required if formaldehyde had not been absorbed.

11.43 c. c. $\frac{n}{10}$ $AgNO_3$ sol. = Quantity combined with KCN.

2.88 c. c. $\frac{n}{10}$ $AgNO_3$ sol. = the formaldehyde absorbed.

1c. c. $\frac{n}{10}$ $AgNO_3$ sol. represents 0.003 gm. formaldehyde.

Then, 0.003 × 2.88 = 0.00864 gm. formaldehyde in 10 liters of air. Therefore, 1 cu. ft. air (28.315 liters) contained 0.0245 gm. formaldehyde.

The titres of the sulphocyanate and cyanide solutions were as follows:

10 c. c. KCNS solution = 10.21 c. c. $\frac{n}{10}$ $AgNO_3$ solution.

10 c. c. KCN solution = 9.54 c. c. $\frac{n}{10}$ $AgNO_3$ solution.

(*b*) *Absorption in water.*—Tubes 1, 2, 3, 4, 5 contained a column of distilled water from 1 to 1.5 inches high. After making connections, air was drawn through them from the room, as already described.

Volume of air drawn from room = 10 liters.
Time required to draw air = 1 hr. 13 min.

Ten c. c. KCN solution were placed in a 300 c. c. beaker and the contents of the five tubes and their connections carefully rinsed into it. This solution was then transferred to a 250 c. c. flask containing 10.1 c. c. $\frac{n}{10}$ $AgNO_3$ solution plus 8 to 10 drops of nitric acid. The flask was filled to the mark with water and thoroughly shaken.

The subsequent procedure is exactly like that given under *a* above, except that not the corrected titre, but the original titre of the cyanide solution is used in the calculation, since the cyanide solution is not subjected to the action of air bubbling through it.

Volume of $\frac{n}{10}$ $AgNO_3$ solution corresponding to formaldehyde absorbed = 3.03 c. c. 3.03 c. c. × 0.003 = 0.00909 gm. formaldehyde in 10 liters of air. One cu. ft. (28.315 liters) air contained 0.0257 gm. formaldehyde.

The quantity of formalin used to charge the room in nearly all the experiments, tabulated further on, was 600 c. c. of 35.66 per cent by volume—i. e., 100 c. c. of the formalin contained 35.66 gm. of absolute formalin. The strength was determined by the Blank and Finkenbeiner method of titration with hydrogen dioxide and caustic-soda solutions.[a] In all the experiments the formalin was taken from the same supply.

In the two experiments just described 600 c. c. of formalin were used to charge the room. The results may be expressed in percentage, based on the amount of absolute formaldehyde taken per cubic foot of space in charging the room. The weight per cubic foot is

---

[a] For description see original article in Berichte, 1898, p. 2979, or the U. S. Pharmacopœia, Eighth Decennial Revision.

$$\frac{600 \text{ c. c.} \times 0.3566}{2000} = 0.107 \text{ gm. HCOH.}$$

In experiment *a* the per cent would be $\frac{0.0245 \times 100}{0.107} = 22.9.$

In *b* it would be $\frac{0.0257 \times 100}{0.107} = 24.01.$

If a correction were not applied in *a* the result would be the same as in *b*.

TEMPERATURE AND HUMIDITY.

In order to determine the temperature and humidity the sling psychrometer was used.[a] This instrument consists of a pair of thermometers fastened to a piece of stiff sheet metal, provided with a handle at the end, by means of which the psychrometer can be rapidly whirled in the air. The bulb of one of the thermometers is covered with clean muslin, which before using is saturated with clean water. These two thermometers are known as the "dry" and "wet" bulb thermometer, respectively. After dampening the wet bulb the psychrometer is rapidly whirled in the air for a few seconds and then the reading of the two thermometers quickly taken. This is repeated until the readings are no longer changed by further whirling. The difference between the readings of the dry and wet bulb thermometers represents the depression of the wet-bulb thermometer, the degree of which is dependent upon the rate of evaporation, which in turn is dependent upon the amount of moisture in the atmosphere. The dry-bulb thermometer represents the temperature of the air.

Knowing the temperature of the air and the depression of the wet-bulb thermometer, the relative humidity and finally the absolute humidity of the air can be ascertained by reference to psychrometric tables for obtaining the vapor pressure, relative humidity, and temperature of the dew-point, prepared by the Weather Bureau, United States Department of Agriculture.[a]

On account of the very important part that humidity plays in formaldehyde disinfection the method of calculating this factor in one case is here given, e. g.: After whirling the psychrometer the dry-bulb thermometer registers 80° F. and the wet-bulb thermometer 73° F. The temperature of the dry-bulb thermometer (80° F.) represents the temperature of the air, and 7° F. represents the difference between the dry and wet bulb thermometers. By referring to the tables for estimating the relative humidity, we find that this difference of 7° F. at a temperature of 80° F. represents a relative humidity of 72 per cent.

---

[a] Marvin, C. F.: Psychrometric tables for obtaining the vapor pressure, relative humidity, and temperature of the dew-point. U. S. Weather Bureau, no. 235, Washington, 1900.

Going further, we find by reference to the tables for estimating the absolute humidity the weight of vapor in a cubic foot corresponding to a temperature of 80° F. and relative humidity of 72 per cent as follows:

	Grains.
At 80° F. and 70 per cent the weight is	7.654
The additional weight for 2 per cent is one-tenth the weight for 20 per cent, viz	.218
Hence the weight at 80° F. and 72 per cent is	7.872

As it was desired to know the temperature, relative and absolute humidity in our work in the room at certain stages of the experiment, and as the presence of the formaldehyde made it impossible to enter the room, the psychrometer was used in another way. After the handle was removed the psychrometer was fastened to a block of wood, which in turn was attached to the end of the shaft of a centrifuge passed through a small hole in one of the doors. By turning the crank of the centrifuge on the outside the psychrometer was rapidly revolved on the inside of the room. A cup of water was provided on the inside, so the wet bulb could be moistened, and a small window in the door just under the revolving apparatus made it possible to read the registration of the thermometers. By means of this apparatus the temperature, humidity, etc., of the room could be determined when desired. The results of whirling the psychrometer by this method were found on comparison to be practically the same as when used by hand.

BACTERIOLOGICAL METHODS.

The nonspore-bearing organisms, *Bacillus pyocyaneous*, *B. coli communis*, *B. typhosus*, *B. dysenteriæ* (Shiga), and *B. diphtheriæ*, were grown on agar slants at temperature of 37° C. for 24 hours before using.

For spores the *B. subtilis* was grown on agar slants about 40 days—a portion of the time at incubator temperature and the other portion at room temperature.

Different kinds of material were tried for exposing the organisms on—such as small pieces of blanket, linen, cotton, silk threads, filter paper, and glass—but it was found that filter paper and glass answered the purpose better than the others, and consequently were used throughout these experiments. It was found that in using a heavy material for this purpose sufficient formaldehyde would at times be carried over into the tube of nutrient bouillon in which they were planted to inhibit bacterial growth. This was especially so if the pieces of material used were of considerable size.

The slips of filter paper and pieces of glass used were about 0.5 cm. square and were sterilized before contaminating with the organism. An emulsion of the organism to be used was made in sterilized water and the slips of filter paper and pieces of glass placed in Petri dishes and saturated with the emulsion. The dishes were then placed in the incubator room at a temperature of 37° C. for one hour, the slips of filter paper and pieces of glass being small, and as they were moved about in the Petri dishes from time to time during the hour this seemed ample time to thoroughly dry them.

The other method used for making the exposures in these experiments is one first used by Doctor Wilson,[a] and which will be called in this work the "Wilson method." As used in these experiments it consisted of a heavy piece of cardboard about 4 by 15 cm. folded in the middle like the cover of a small book and having a small piece of filter paper about 0.5 by 4 cm. pasted by one end inside of it something like the leaf of a book. This left the other end of the filter paper unattached. This end was contaminated with the organism to be exposed.

A small nick in the filter paper about 0.5 cm. from this end facilitated tearing off the end with forceps when so desired. A number of these little devices were prepared in this way and sterilized in a hot-air sterilizer. The free ends of the filter paper were moistened with a watery suspension of the organism and dried in the incubator room for one hour as with the other methods above described.

The organisms were then ready to be exposed to the action of the formaldehyde gas.

The exposures were made in the wire trays in the room which have been described.

The trays, being immovable, were easily sterilized in a gas flame. The slips of filter paper and pieces of glass were carefully placed in the trays by means of sterile forceps, care being taken not to have any two pieces in contact with each other.

The exposures with the Wilson method were also made on these trays.

As no appreciable difference in the germicidal action of the formaldehyde on exposures made at different heights in the room was observed, most of the exposures, for convenience, were made through the middle series of zinc tubes.

After the organism had been exposed to the action of the formaldehyde the desired time, the rubber stopper was removed and the wooden frame carrying the wire tray momentarily withdrawn far enough to procure one of the exposures.

The slips of filter paper and pieces of glass were removed with sterile forceps and planted in tubes of nutrient bouillon. With the Wilson method the slips of filter paper were torn off with sterile forceps and planted in the same way.

---

[a] Robert J. Wilson, M. D., assistant director of the Research Laboratory, New York City Department of Health.

In this manner plants into tubes of nutrient bouillon were made at different intervals when so desired. The tubes were placed in the incubating room at a temperature of 37° C. and the results recorded after ten days' incubation.

Other methods of exposing organisms, such as placing slips of contaminated filter paper in envelopes and in small paper boxes in which numerous holes had been punched, were tried for the purpose of testing the penetrating power of formaldehyde, but the results were so unsatisfactory that the methods were abandoned.

The Wilson method affords a very simple and convenient way of exposing organisms to the action of a gaseous disinfectant, and in case it is impracticable to make the plants immediately after making the exposures they can be taken to the laboratory and planted without much risk of outside contamination.

DISINFECTANTS AUTHORIZED BY U. S. QUARANTINE REGULATIONS AND THE PROPER METHODS OF GENERATING AND USING SAME.

FORMALDEHYDE GAS.

163. Formaldehyde gas is effective if applied by one of the methods given below. Formaldehyde gas has the advantage as a disinfectant that it does not injure fabrics or most colors. It is not poisonous to the higher forms of animal life. It fails to kill vermin such as rats, mice, roaches, bedbugs, etc. The method is not applicable to the holds of large vessels. Formaldehyde is applicable to the disinfection of rooms, clothing, and fabrics, but should not be depended upon for bedding, upholstered furniture, and the like, when deep penetration is required.[a]

164. Many formaldehyde solutions do not contain 40 per cent of formaldehyde, and all are apt to deteriorate with time. It is therefore necessary to use a quantity in excess of the amount prescribed in these regulations, unless the solution has been recently analyzed.

165. The following methods of evolving the gas may be used:

(*a*) Autoclave under pressure, 3 to 12 hours' exposure.

(*b*) Lamp or generator, 6 to 18 hours' exposure.

(*c*) Spraying, 12 to 24 hours' exposure.

(*d*) Formaldehyde and dry heat in partial vacuum, 1 hour's exposure.

166. The minimum number of hours' exposure as given above applies to empty rooms of tight construction containing smooth, hard surfaces; the maximum number of hours' exposure applying in all cases to textiles and other articles of a similar kind requiring more or less penetration.

167. Autoclave under pressure. This method has considerable penetrating power when applied as detailed below. Rooms or apartments

---

[a] It should be noted that formaldehyde disinfection is more efficient in warm, moist, or still weather than in cold, dry, or windy weather.

need no special preparation beyond the ordinary closing of doors and windows. Pasting, caulking, or chinking of ordinary cracks and crevices is not necessary. The doors of lockers and closets and the drawers of bureaus should be opened. In this apparatus use formalin (40 per cent), with the addition of a neutral salt, such as calcium chloride (20 per cent). The gas must be evolved under a pressure not less than 45 pounds. After the gas is separated from its watery solution the pressure may be allowed to fall and steam projected into the compartment to supply the necessary moisture. Use not less than 10 ounces of formalin per 1,000 cubic feet, and keep the room closed for three to twelve hours after the completion of the process. For large rooms the gas must be introduced at several points as far apart as possible. It is applicable to the disinfection of clothing and fabrics suspended loosely in such a manner that every article is freely accessible to the gas from all directions.

168. Lamp or generator. This method requires an apparatus producing formaldehyde by a partial oxidation of wood alcohol, and in using it the room or apartment should be rendered tight as practicable. Oxidize 24 ounces of wood alcohol per 1,000 cubic feet, and keep the room closed for six to eighteen hours, in accordance with the provisions of paragraph 165. This method leaves little or no odor. When applied to clothing and textiles, the articles should be suspended in a tight room and so disposed as to permit free access of the gas. (See also Par. 166.) The wood alcohol should be of 95 per cent strength, and should not contain more than 5 per cent of acetone.

169. Spraying. The formalin (40 per cent) should be sprayed on sheets suspended in the room in such a manner that the solution remains in small drops on the sheet. Spray not less than 10 ounces of formalin (40 per cent) for each 1,000 cubic feet. Used in this way a sheet will hold about 5 ounces without dripping or the drops running together. The room must be very tightly sealed in disinfecting with this process, and kept close not less than twelve hours. The method is limited to rooms or apartments not exceeding 2,000 cubic feet. The formalin may also be sprayed upon the walls, floors, and objects in the rooms.

170. Formaldehyde with dry heat in partial vacuum. This method has superior penetrating powers and is specially applicable to clothing and baggage. The requirements of this method are (1) dry heat of 60° C. sustained for one hour; (2) a vacuum of 15 inches; (3) formaldehyde evolved from a mixture of formalin with a neutral salt, in an autoclave under pressure, using not less than 30 ounces of formalin (40 per cent) for 1,000 cubic feet; and (4) a total exposure, under these combined conditions, of one hour.

171. The stated times of exposure to sulphur dioxide and formaldehyde are sufficient to destroy bacterial infection due to non-spore-

bearing organisms, providing that the infection is present on the surface. If the room is of peculiar construction, so as to impede the diffusion of the gas, or if the room is a dirty one, or if on account of any other condition rendering the germicidal action of the gas more difficult, the time of exposure should be proportionately increased, or supplanted by other methods.

DIFFERENT METHODS OF EVOLVING FORMALDEHYDE GAS.[a]

I. FORMALIN-PERMANGANATE METHOD.

When formalin is poured upon crystals of potassium permanganate, a vigorous reaction takes place, accompanied by strong ebullition of the liquid and sufficient heat to produce a large quantity of formaldehyde gas, water vapor, etc. The time required for the reaction to begin, or at least to become apparent, varies from a few seconds to a minute or two, depending upon the temperature.

The reaction is apparently over in a comparatively short time (about five minutes), and with proper proportion of substances the residue in the vessel is almost dry. Evans and Russell used the proportion of 100 c. c. of formalin to 37.5 grams of permanganate, but we found that with this proportion considerable formalin remained in the residue, as was evidenced by its wet condition and powerful odor of formaldehyde. After some experimenting we adopted the proportion of 100 c. c. of formalin to 50 grams of permanganate, which gave a residue fairly free from liquid.

The quantity used to charge the room of 2,000 cubic feet was in nearly all cases 600 c. c. of formalin (containing 35.66 per cent of formaldehyde by volume). The generator was a galvanized-iron pail 10 inches in diameter and 10 inches deep, holding 12 liters (3 gallons). The pail was placed in the center of the room, the permanganate dropped into it and the formalin quickly poured upon it. The ebullition was so vigorous that the frothy mass often reached nearly to the top of the pail.

We did not investigate the exact nature of the chemical reaction that took place, as for our purposes we were only interested in the fact that an abundance of formaldehyde was given off in gaseous form. According to Evans and Russell, "analysis of the gas thrown out into a room by this reaction showed it to consist of formaldehyde, water vapor, carbon dioxide, and a very small amount of formic acid. In the generator were found a lower oxide of manganese, a little formaldehyde, carbon dioxide, potassium hydroxide, and, I think, a little potassium formate resulting from the neutralization of the potassium hydrate by formic acid." It seems highly probable that the decomposition takes place essentially according to the following reaction: $4KMnO_4 + 3HCHO + H_2O = 4MnO(OH)_2 + 2K_2CO_3 + CO_2$ (CO_2), and that the heat resulting from this reaction causes the liberation of formaldehyde gas from the remaining portion of the formalin.

---

[a] In the experiments which follow, the quantities of formalin employed for the evolution of formaldehyde gas have been in strict accordance with the U. S. Quarantine regulations. In the case of the formaldehyde generating lamp, the quantities of methyl alcohol used have been less than those prescribed by the regulations; about 10 per cent less in the experiments in Pullman cars and about 30 per cent less in the room experiments.

The reaction is more rapid the more finely powdered the permanganate crystals are, but in our experiments we used the small needle crystals of commerce without further powdering.

The temperature and relative humidity of the air in the room were recorded before as well as at definite intervals after mixing the formalin and permanganate. This showed a considerable amount of moisture given off by the process.

Many variations and conditions might have been tried, such as covering the pail with sheet asbestos to retain the heat, heating the pail before the experiment or placing it in hot water, powdering the permanganate crystals, etc., but as time is a factor we adopted those conditions which are least difficult to execute in disinfecting rooms.

Before a new charge was put into the room all formaldehyde of the previous experiment was got rid of by opening the doors and windows until no odor of formaldehyde was perceptible.

Chemical determinations.—In making these determinations approximately fifteen minutes were allowed to elapse after mixing the formalin and permanganate before beginning to draw air from the room. This seemed ample time for the reaction to cease and for the formaldehyde gas to diffuse through the air of the room. The results are shown in the following table:

TABLE 1.—*Experiments with the formalin-permanganate method.*

Number.	Temperature of room before experiment.	Formalin (35.66 per cent by volume).	$KMnO_4$.	Time between mixing and drawing air.	Time required to draw air.	Volume of air drawn, in liters.	Relative humidity before experiment.	Relative humidity 10 minutes after mixing.	Increase in grains of moisture per cubic foot.	Abs. formaldehyde used per cubic foot.	Abs. formaldehyde found per cubic foot.	Per cent yield.	Condition of wind, etc.
	°*F.*	*c. c.*	*gm.*	*h. m.*	*h. m.*					*gm.*	*gm.*		
I	71	600	300	*a* 14	1 10	10	72	86	1.47	0.107	0.04035	37.70	Moderate wind.
				b 3 38	1 21	10	....	....	......	.107	.0243	22.7	Do.
II	69	800	400	*a* 12	1 32	10	63	86	2.0	.1426	.0505	35.41	Slight breeze.
				b 22 17	1 10	11	....	....	......	.1426	.01722	12.07	Very slight breeze.
III	77	400	200	15	1 12	10	79	85	0.58	.0713	.025	35.06	Moderate wind.
IV	79	600	300	17	1 21	10	79	94	1.72	.107	.0419	39.15	Practically no wind; windows and doors of room pasted up.
V	62	600	300	15	1 15	10	55	74	1.17	.107	.02685	25.1	No breeze.
VI	52	600	300	15	1 20	10	63	78	.54	.107	.01186	11.09	No wind.

Although the room in which the experiments were made was zinc-lined and the doors and windows were fairly close fitting, still it was not air-tight, so that the conditions were not of that exact quantitative character as obtains in an operation like the precipitation of sulphuric acid by barium chloride. In view of this condition of the room, which, of course, allowed the winds to exert a varying influence upon the rate of leakage therefrom, the percentages obtained in those experiments performed under approximately the same conditions as to temperature can be considered as agreeing fairly well. The results also indicate that the relation between the formalin and permanganate in the ratio taken is fairly quantitative and that the percentage yield of formaldehyde gas is approximately the same whether 400, 600, or 800 c. c. be taken for a charge. Experiments Ib and IIb table give an idea of the rate of leakage of formaldehyde from the room. In III, where the room was made more air-tight by pasting paper over the cracks of the door and covering the window frames, the per cent is a little higher.

The percentage yield of formaldehyde with this method is very much affected by certain variations in the temperature. This, however, is not the case with experiments done at temperatures above 60° to 65° F. The results then agree rather closely, and it is probable that with this condition of temperature on a very quiet day with a charge of 600 c. c. formalin (35.66 per cent by volume) the weight of the gas in the room would be between 38 and 39 per cent of the weight in the 600 c. c. of formalin used. This percentage is only slightly less than that obtained under similar conditions with the retort and autoclave methods.

Effects of low temperature upon the state of the formaldehyde in the room.—Provided the temperature is below 60° or 65° F., the liberation of formaldehyde by this method produces a hazy or foggy condition of the air in the room. This is most marked just after the process of liberation is complete; but it does not entirely disappear during the time required to do an experiment, usually three hours. The lower the temperature in the room the more marked is this condition, which apparently is due to some change in the formaldehyde, resulting from the effects of cold upon it and to a certain extent to the presence of the aqueous vapor liberated. The latter, however, would soon become invisible.

The exact change that takes place in the formaldehyde is not plain, but evidently there is much polymerization, as is shown by the paraform deposited in the room after the experiment is completed.

The hazy condition in the room is about the same with the formalin-permanganate method as with the retort and autoclave; but the deposit of paraform is more noticeable with the former than the latter two methods.

This change of state in the formaldehyde, of course, very materially reduces the percentage obtained in our determinations, as is shown in experiments VI and VII, table 1. In VI, as the temperature (62° F.) was close to the point where polymerization seems to begin, 25.1 per cent was found; but in VII (temperature 52° F.) only 11.09 per cent was obtained.

These results can not be interpreted as positively representing the percentage of gas actually existing as such in the air during the time the determinations were made, since it is possible that all the paraform or other changed formaldehyde was not eliminated from the determinations, although a small pledget of cotton was placed in the funnel of the drawing tube to prevent the paraform, etc., from passing out in the air drawn. The cotton pledget was placed in the funnel of the drawing tube only when working at a reduced temperature and when some polymerization was apparent.

Germicidal properties.—For the purpose of determining the germicidal properties of formaldehyde liberated by this method the organisms were placed in the wire trays in the room before mixing the formalin and permanganate, and the time of exposure counted from the time the reaction and liberation of the formaldehyde began.

As most of the gas is given off in the first minute or two, the reason for this is evident.

The method of preparing the organisms, making the exposures, etc., have been described. A summary of conditions and results is given in each of the following experiments:

EXPERIMENT NO. 1.

Formalin-permanganate method.

Formalin, 400 c. c.
Permanganate, 200 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.0713 gram.
Quantity of formaldehyde found per cubic foot, 0.025 gram.

(Determination made by drawing air from the room, beginning 15 minutes after mixing the formalin and permanganate, and consuming 1 hour 12 minutes in the drawing.)

Yield of formaldehyde, 35.06 per cent.
Weather: Moderate breeze from west; almost clear.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before mixing $HCHO$ and $KMnO_4$	77	79	7.88
After 10 minutes	77	85	8.46
After 2 hours	78	79	8.12

NOTE.—In all the experiments with this method the temperature, humidity, and time of exposure of organisms are given from the moment of mixing the formalin and permanganate.

EXPERIMENT NO. 1—Continued.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		5	15	30	45	60	120
B. pyocyaneus	Filter paper	–	–	–	–	–	–
B. coli communis	do	–	–	–	–	–	–
B. dysenteriæ	do	+	–	–	–	–	–
B. subtilis	do	+	+	–	–	–	–
B. coli communis	Wilson method	+	+	+	+	–	–
B. subtilis	do	+	+	+	+	+	–

EXPERIMENT NO. 2.

Formalin-permanganate method.

Formalin, 600 c. c.
Permanganate, 300 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.0419 gram.

(Determination made by drawing air from the room, beginning 17 minutes after mixing the formalin and permanganate, and consuming 1 hour 21 minutes in the drawing.)

Yield of formaldehyde, 39.15 per cent.
Weather: Practically no wind.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before mixing HCHO and $KMnO_4$	79	79	8.39
After 10 minutes	79.5	94	10.10
After 1 hour	79.5	89	9.57

See Note, Experiment No. 1.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		5	10	15	20	30	45	60
B. pyocyaneus	Filter paper	–	–	–	–	–	–	–
B. coli communis	do	–	–	–	–	–	–	–
B. dysenteriæ	do	+	–	–	–	–	–	–
B. subtilis	do	+	+	+	+	....	–	–
B. coli communis	Wilson method	+	+	–	–	–	–	–
B. subtilis	do	+	+	+	+	+	+	–

EXPERIMENT NO. 3.

Formalin-permanganate method.

Formalin, 600 c. c.
Permanganate, 300 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.0353 gram.

(Determination made by drawing air from the room, beginning 15 minutes after mixing the formalin and permanganate and consuming 1 hour 15 minutes in the drawing.)

Yield of formaldehyde, 33 per cent.
Weather: Strong wind from the southwest; clear.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before mixing HCHO and $KMnO_4$	77.5	72	7.28
After 10 minutes	78.0	87	8.94
After 1 hour	78.0	81	8.30

See Note, Experiment No. 1.

[+ means growth; − no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		5	10	15	20	30	45	60
B. pyocyaneus	Filter paper	+	+	−	−	−	−	−
B. coli communis	do	+	−	−	−	−	−	−
B. dysenteriæ	do	+	+	−	−	−	−	−
B. subtilis	do	+	+	+	....	−	−	−
B. coli communis	Wilson method	+	+	−	−	−	−	−
B. subtilis	do	+	+	+	+	+	+	−

EXPERIMENT NO. 4.

Formalin permanganate method.

Formalin, 600 c. c.
Permanganate, 300 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde liberated, undetermined.
Weather: Cloudy; light westerly winds.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before mixing HCHO and $KMnO_4$	78	75	7.70
After 10 minutes	78	91	9.34
After 2 hours	78	91	9.34

See Note, Experiment No. 1.

EXPERIMENT NO. 4—Continued.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.				
		5	10	20	30	60
B. coli communis	Glass	–	–	–	–	–
B. subtilis	do	...	...	...	–	–
B. pyocyaneus	Filter paper	–	–	–	–	–
B. typhosus	do	+	–	–	–	–
B. coli communis	do	+	–	–	–	–
B. subtilis	do	...	...	...	–	–
B. coli communis	Wilson method	+	–	–	–	–

The minimum temperature and relative humidity in the foregoing experiments were 77° F. and 72 per cent, respectively; under these conditions the germicidal results were very satisfactory. The non-spore-bearing organisms exposed on slips of filtered paper and pieces of glass were killed within a few minutes. The spores of *B. subtilis* exposed in the same manner were likewise killed within a comparatively short time. The time required to kill the same organisms exposed by the Wilson was more than twice as long as that required with the other methods of exposure.

Although only 400 c. c. of formalin were used in Experiment No. 1, the results are about the same as with the other experiments in which 600 c. c. were used. Comparing this same experiment (No. 1) with the following one (No. 5) in which 800 c. c. of formalin were used, the germicidal results of exposures on filter paper are in favor of the former experiment, due to the higher temperature and humidity in this experiment; the results of exposures by the Wilson method are in favor of the latter experiment, due in all probability to the increased percentage of formaldehyde in the air, favoring penetration.

EXPERIMENT NO. 5.

Formalin-permanganate method.

Formalin, 800 c. c.
Permanganate, 400 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.1426 gram.
Quantity of formaldehyde found per cubic foot, 0.0505 gram.
(Determination made by drawing air from the room, beginning 12 minutes after mixing the formalin and permanganate, and consuming 1 hour 32 minutes in the drawing.)
Yield of formaldehyde, 34.41 per cent.
Weather: Light, westerly wind; almost clear.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before mixing HCHO and $KMnO_4$	69	63	4.86
After 10 minutes	70	86	6.86
After 1 hour	70	81	6.46

See Note, Experiment No. 1.

EXPERIMENT NO. 5—Continued.

[+ means growth; — no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		5	10	20	30	40	60
B. pyocyaneus	Filter paper	+	—	—	—	—	—
B. typhosus	do	+	+	—	—	—	—
B. coli communis	do	+	+	+	—	—	—
B. dysenteriæ	do	+	+	....	—	—	—
B. coli communis	Wilson method	+	+	+	....	—	—
B. subtilis	do	+	+	+	+	....	—

EXPERIMENT NO. 6.

Formalin-permanganate method.

Formalin, 600 c. c.
Permanganate, 300 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.04035 gram.

(Determination made by drawing air from the room, beginning 14 minutes after mixing the formalin and permanganate, and consuming 1 hour 10 minutes in the drawing.)

Yield of formaldehyde, 37.70 per cent.
Weather: Moderate northwest wind, clear.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before mixing HCHO and $KMnO_4$	71	45	3.70
After 10 minutes	72	60	5.10
Alter 20 minutes	72	58	4.93
After 2 hours	72	55	4.68

See Note, Experiment No. 1.

[+ means growth; — no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		5	10	20	45	60	90	120
B. coli communis	Glass	+	+	+	—	—	—	—
B. pyocyaneus	Filter paper	+	+	+	+	—	—	—
B. typhosus	do	+	+	+	+	—	—	—
B. coli communis	do	+	+	+	+	+	—	—
B. dysenteriæ	do	+	+	+	+	—	—	—
B. coli communis	Wilson method	+	+	+	+	+	+	—
B. subtilis	do	+	+	+	+	+	+	+

The preceding experiment (No. 6) shows the effects of a relatively low humidity upon the germicidal power of the formaldehyde. Although the temperature in this experiment was comparatively high the time required to kill was much prolonged as compared with the experiments in which the temperature and humidity were both high.

The time to kill is comparatively longer with the Wilson method of making exposures than with the other methods; this fact may be attributed to the lower humidity diminishing the power of penetration of the formaldehyde.

The following two experiments show the inefficient results obtained when the temperature is comparatively low. No doubt slightly better results would have been obtained in these two experiments if the humidity had been higher, say 90 per cent. In Experiment No. 7, temperature 62° F., some polymerization of the formaldehyde took place, as was evidenced by the hazy appearance of the air in the room and the decreased percentage of formaldehyde obtained; but it was not nearly so marked as in Experiment No. 8, temperature 52° F. In the latter experiment quite a deposit of paraform was observed in the room after opening.

EXPERIMENT NO. 7.

Formalin-permanganate method.

Formalin, 600 c. c.
Permanganate, 300 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.02685 gram.
(Determination made by drawing air from the room, beginning 15 minutes after mixing the formalin and permanganate and consuming 1 hour 15 minutes in the drawing.)
Yield of formaldehyde, 25.1 per cent.
Weather: Cloudy, no wind.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before mixing HCHO and $KMnO_4$	62	55	3.37
After 10 minutes	62	74	4.54
After 3 hours	62	64	3.93

See note, Experiment No. 1.

[+ means growth; — no growth.]

Organism.	How exposed	Time of exposure in minutes, and results.							
		10	20	30	45	60	90	120	180
B. coli communis	Filter paper	+	–	–	–	–	–	–	–
B. typhosus	do	–	–	–	–	–	–	–	–
B. subtilis	do	+	+	+	+	–	–	–	–
B. coli communis	Wilson method	+	+	+	+	+	+	–	–
B. subtilis	do	+	+	+	+	+	+	+	+

EXPERIMENT NO. 8.

Formalin-permanganate method

Formalin, 600 c. c.
Permanganate, 300 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.01186 gram.

(Determination made by drawing air from the room, beginning 15 minutes after mixing the formalin and permanganate and consuming 1 hour 20 minutes in the drawing.)

Yield of formaldehyde, 11.09 per cent.
Weather: Rainy, very little wind.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before mixing HCHO and $KMnO_4$	52	63	2.75
After 10 minutes	51	78	3.29
After 3 hours	50	71	2.89

See note, Experiment No. 1.

[+ means growth; − no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.							
		10	20	30	45	60	90	120	180
B. coli communis	Filter paper	+	+	+	−	−	−	−	−
B. typhosus	do	+	+	−	−	−	−	−	−
B. subtilis	do	+	+	+	+	+	+	+	−
B. coli communis	Wilson method	+	+	+	+	+	+	+	+
B. subtilis	do	+	+	+	+	+	+	+	+

The results with the formalin-permanganate method are all that can be desired for practical disinfection purposes, provided the temperature and relative humidity are comparatively high. Under these conditions no particular advantage is gained, especially for surface disinfection, by having a large quantity of formaldehyde gas present, as is shown by the above experiments in which different quantities of formalin were used. With a low temperature or humidity formaldehyde is practically useless as a disinfectant.

The minimum percentage of humidity and lowest temperature at which disinfection with formaldehyde can be accomplished can not be stated accurately, as the one depends to a limited extent upon the other. However, it seems that for practical disinfection with the formalin-permanganate method the humidity should not be below 60 or 65 per cent and the temperature not below about 65° F. The former refers to the percentage of humidity in the air of the room before starting an experiment. In our experiments the humidity in the air of the room was increased 15 to 23 per cent in the different experiments by the moisture given off from the formalin-permanganate. Just what part this increase in humidity plays in disinfecting with formaldehyde can not be stated exactly; but the indications are that too much dependence must not be placed in it. No doubt it is an advantage, but it does not seem to answer the purpose so well as the natural humidity of the atmosphere.

The special points of advantage of the formalin-permanganate method of evolving formaldehyde are that it is simple in operation, no special apparatus is required, it is economical both as to time and money, it produces considerable moisture, and it liberates a large quantity of formaldehyde in a relatively short time. This last fact is of special advantage, since with an ordinary room no pasting of cracks, etc., is required. For disinfection purposes it is better to have a large quantity of formaldehyde with a short exposure than a small quantity with a long exposure. A large quantity of formaldehyde and a high temperature and humidity all assist penetration.

II. DILUTED FORMALIN AND PERMANGANATE METHOD.

A number of experiments were made with formalin diluted with various quantities of water and different weights of permanganate.

The purpose in adding the water was to increase, if possible, the amount of moisture given off.

In each experiment 600 c. c. of formalin were used, to which were added, before mixing with the permanganate, 300 c. c. of water in some of the experiments and 600 c. c. in the others.

For every 100 c. c. of water used an additional 25 grams of permanganate were required in order to obtain a residue fairly free from liquid.

The time required for the reaction to begin was slightly diminished in this method as compared with the undiluted formalin-permanganate method. The ebullition, too, seemed to be more violent.

Except for the differences above noted, the method of procedure in charging the room was the same as with the undiluted formalin-permanganate method already described.

Chemical determinations. The interval between setting off the charge and beginning to draw air, the volume of air drawn (10 liters) and the time required to draw it were about the same as in Table 1. In several instances two experiments were made simultaneously with 5 and 10 liters of air respectively.

The results are shown in the following table:

TABLE 2.—*Experiments with the diluted formalin-permanganate method.*

Number.	Temperature of room before experiment.	Formalin (35.66 per cent by volume).	Water.	$KMnO_4$.	Time between mixing and drawing air.	Time required to draw air.	Volume of air drawn, in liters.	Relative humidity before experiment.	Relative humidity 10 minutes after mixing.	Increase in grains of moisture per cubic foot.	Abs. formaldehyde used per cubic foot.	Abs. formaldehyde found per cubic foot.	Per cent yield.	Condition of wind, etc.
	°*F.*	*c. c.*	*c. c.*	*gm.*	*h. m.*	*h. m.*					*g. m.*	*g. m.*		
I	81	600	600	450	17 0	*a* 1 20	10	81	94	1.79	0.107	0.0272	25.4	Slight breeze.
						b 1 20	10	....	....	......	.107	.0272	25.4	Do.
II	81	600	300	450	16 0	1 5	10	83	98	2.06	.107	.03081	28.8	Moderate wind.
III	81.5	600	300	300	16 0	*a* 1 9	5	74	94	2.28	.107	.0242	22.6	Do.
						b 1 8	10	....	....	......	.107	.02336	21.8	Do.
IV	81	600	300	375	14 0	*a* 0 45	5	77	96	2.14	.107	.0289	27	Brisk wind.
						b 1 14	10	....	....	......	.107	.02965	27.7	Do.
V	85	600	300	375	15 0	*a* 0 34	5	70	90	2.55	.107	.03755	35.1	Gentle breeze.
						b 1 5	10	....	....	......	.107	.0356	33.27	Do.
VI	85	600	300	375	*a* 0 17	0 42	5	70	88	2.3	.107	.0341	31.84	Do.
					b 0 17	1 20	10	....	....	......	.107	.03296	30.80	Do.
					c 2 27	1 18	10	....	....	......	.107	.02548	23.81	Do.
					d 4 24	1 0	10	....	....	......	.107	.02276	21.27	Do.
					e 24 33	1 23	10	....	....	......	.107	.01028	9.6	Do.
VII	79	600	300	375	*a* 0 21	0 47	5	79	94	1.72	.107	.03517	32.87	Practically no wind; windows and doors pasted up with paper.
					b 0 21	1 17	10	....	....	......	.107	.03517	32.87	
					c 4 7	1 18	10	....	....	......	.107	.02658	24.84	
					d 23 46	1 13	10	....	....	......	.107	.01546	14.44	
VIII	39	600	300	375	0 15	1 5	10	51	92	.54	.107	.01626	15.2	Moderate wind.
IX	41	600	600	450	0 15	1 30	11	55	92	.89	.107	.01334	11.53	Do.

Of the various proportions used that of 600 c. c. formalin, 300 c. c. water, and 375 gms. of permanganate gives the highest yield of formaldehyde gas.

In experiment I the determinations *a* and *b*, made simultaneously in the same time and on the same volume of air, gave the same results; this would seem to indicate that the method of analysis, under the same conditions, gives constant results. In experiment III 5 liters of air in *a* were drawn in the same time as 10 liters in *b*, with practically the same result; this seems to point to the conclusion that 5 liters of air is a large enough quantity for a determination.

In experiments IV, V, VI, and VII, the same proportions of materials were used to charge the room, but the results in IV are low on account of the brisk wind blowing at the time and should not be compared with those of V, VI, and VII. The three determinations of V, VI, and VII, which are comparable, show a fairly close agreement. VI and VII show the rate of leakage from the room, and *d* and *e* of VI and *c* and *d* of VII, which are comparable, show that when there is a very little wind the leakage from the room when pasted up with paper is not much less than when not so pasted.

A reduction in temperature produces about the same hazy appearance in the room that is observed in case of the undiluted formalin-permanganate, retort, and autoclave methods, but the deposit of paraform is less than in the undiluted formalin-permanganate method. This result may be due to the decreased percentage of formaldehyde which is given off or to the decreased polymerization attendant upon the amount of water added. The percentages obtained by the diluted formalin-permanganate method are relatively higher, considering the temperatures, than is the case with the undiluted formalin-permanganate method, as will be seen by comparing experiments VIII and IX, Table 2, with VI and VII, Table 1. The difference in percentage obtained in experiments VIII, temperature 39° F. (15.2 per cent) and IX, temperature 41° F. (11.53 per cent), Table 2, is probably due to the larger quantity of water added in the latter experiment.

Germicidal properties.—The method of procedure was the same as with the undiluted formalin-permanganate method already described. A summary of conditions and results is given in each of the following experiments:

EXPERIMENT NO. 9.

Dilute formalin-permanganate method.

Formalin, 600 c. c.
Water, 600 c. c.
Permanganate, 450 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.0272 gram.
(Determination made by drawing air from the room, beginning 17 minutes after mixing the formalin and permanganate, and consuming 1 hour 20 minutes in the drawing.)
Yield of formaldehyde, 25.4 per cent.
Weather: Light northerly breeze, clear.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before mixing HCHO and $KMnO_4$	81	81	9.13
After 10 minutes	82	94	10.92
After 2 hours	82.5	90	10.62

NOTE. In all the experiments with this method the temperature, humidity, and time of exposure of organisms are given from the moment of mixing the formalin and permanganate.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		5	10	25	45	60	120
B. pyocyaneus	Filter paper	+	–	–	–	–	–
B. coli communis	do	+	–	–	–	–	–
B. dysenteriæ	do	+	+	–	–	–	–
B. subtilis	do	+	+	+	+	–	–
B. coli communis	Wilson method	+	+	–	–	–	–
B. subtilis	do	+	+	+	+	+	–

EXPERIMENT NO. 10.

Dilute formalin-permcnganate method.

Formalin, 600 c. c.
Water, 600 c. c.
Permanganate, 450 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.0279 gram.

(Determination made by drawing air from the room, beginning 16 minutes after mixing the formalin and permanganate, and consuming 1 hour 12 minutes in the drawing.)

Yield of formaldehyde, 26.1 per cent.
Weather: Light east wind, cloudy.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before mixing HCHO and $KMnO_4$	80	74	8.09
After 10 minutes	81	91	10.82
After 2 hours	81	91	10.26

See note, Experiment No. 9.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		5	10	20	30	60	120
B. coli communis	Glass	–	–	–	–	–	–
B. subtilis	do	....	....	....	–	–	–
B. pyocyaneus	Filter paper	–	–	–	–	–	–
B. dysenteriæ	do	+	–	–	–	–	–
B. coli communis	do	+	–	–	–	–	–
B. subtilis	do	....	....	....	–	–	–
B. coli communis	Wilson method	+	+	–	–	–	–

EXPERIMENT NO. 11.

Dilute formalin-permanganate method.

Formalin, 600 c. c.
Water, 300 c. c.
Permanganate, 375 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.03517 gram.

(Determination made by drawing air from the room, beginning 21 minutes after mixing the formalin and permanganate, and consuming 47 minutes in the drawing.)

Yield of formaldehyde, 32.87 per cent.
Weather: Light breeze, cloudy.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before mixing HCHO and $KMnO_4$	79	79	8.38
After 10 minutes	79.5	94	10.10
After 2 hours	80	89	9.57

See note, Experiment No. 9.

EXPERIMENT NO. 11—Continued.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.				
		5	10	20	30	60
B. pyocyaneus	Filter paper	+	–	–	–	–
B. coli communis	do	+	–	–	–	–
B. dysenteriæ	do	+	–	–	–	–
B. subtilis	do	+	+	+	+	–
B. coli communis	Wilson method	+	+	–	–	–
B. subtilis	do	+	+	+	+	–

EXPERIMENT NO. 12.

Dilute formalin-permanganate method.

Formalin, 600 c. c.
Water, 300 c. c.
Permanganate, 375 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.0289 gram.

(Determination made by drawing air from the room, beginning 14 minutes after mixing the formalin and permanganate, and consuming 45 minutes in the drawing.)

Yield of formaldehyde, 27 per cent.
Weather: Moderate east wind, rainy.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before mixing HCHO and $KMnO_4$	81	77	8.26
After 10 minutes	81	96	10.82
After 2 hours	82	88	10.23

See note, Experiment No. 9.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.				
		5	10	30	60	120
B. pyocyaneus	Filter paper	+	–	–	–	–
B. coli communis	do	+	+	–	–	–
B. dysenteriæ	do	+	–	–	–	–
B. subtilis	do	+	+	+	–	–
B. coli communis	Wilson method	+	+	–	–	–
B. subtilis	do	+	+	+	+	+

EXPERIMENT NO. 13.

Dilute formalin-permanganate method.

Formalin, 600 c. c.
Water, 300 c. c.
Permanganate, 375 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.03755 gram.

(Determination made by drawing air from the room, beginning 15 minutes after mixing the formalin and permanganate, and consuming 34 minutes in the drawing.)

Yield of formaldehyde, 35.1 per cent.
Weather: Moderate breeze from west, few clouds.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before mixing HCHO and $KMnO_4$	85	70	8.91
After 10 minutes	85	90	11.21
After 2 hours	85	82	10.43

See note, Experiment No. 9.

[+ means growth; — no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		5	10	25	30	60	120
B. pyocyaneus	Filter paper	—	—	—	—	—	—
B. coli communis	do	—	—	—	—	—	—
B. dysenteriæ	do	+	—	—	—	—	—
B. subtilis	do	+	+	+	+	—	—
B. coli communis	Wilson method	+	+	—	—	—	—
B. subtilis	do	+	+	+	+	+	—

EXPERIMENT NO. 14.

Dilute formalin-permanganate method.

Formalin, 600 c. c.
Water, 300 c. c.
Permanganate, 375 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.0341 gram.

(Determination made by drawing air from the room, beginning 17 minutes after mixing the formalin and permanganate, and consuming 42 minutes in the drawing.)

Yield of formaldehyde, 31.84 per cent.
Weather: Very light breeze, clear.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before mixing HCHO and $KMnO_4$	85	70	8.91
After 10 minutes	85	88	11.21
After 2 hours	86	83	10.89

See note, Experiment No. 9.

EXPERIMENT NO. 14—Continued.

[+ means growth, – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		5	10	15	30	60	120
B. pyocyaneus	Filter paper	+	–	–	–	–	–
B. coli communis	do	–	–	–	–	–	–
B. dysenteriæ	do	+	–	–	–	–	–
B. subtilis	do	+	+	+	–	–	–
B. coli communis	Wilson method	+	+	–	–	–	–
B. subtilis	do	+	+	+	+	–	–

The organisms were killed in the above experiment in about the same time as in those with the undiluted formalin-permanganate method under similar conditions of temperature and humidity.

The following two experiments (Nos. 14a and 14b), which were done after the room in experiment No. 14 had been left closed 4 and 24 hours, respectively, show how the percentage of formaldehyde became reduced by standing.

Although only 9.6 per cent of formaldehyde was found in experiment No. 14b, the organisms were killed within a comparatively short time. This demonstrates what a small percentage of formaldehyde will kill when the temperature and humidity are high. Under these conditions the principal effect of a limited reduction in the percentage of formaldehyde seems to be in decreasing penetration, as shown by results with exposures by the Wilson method.

EXPERIMENT NO. 14a.

After the room in experiment No. 14 had been left closed 4 hours.

Quantity of formaldehyde found per cubic foot, 0.02276 gram.
Yield of formaldehyde, 21.27 per cent.
Weather: Very gentle breeze during intervening 4 hours.
Temperature of room during experiment, 87° F.
Relative humidity, 79 per cent.
Absolute humidity, 10.69 grams per cubic foot.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		5	10	25	30	45	60	120
B. pyocyaneus	Filter paper	+	+	–	–	–	–	–
B. coli communis	do	+	–	–	–	–	–	–
B. dysenteriæ	do	+	+	–	–	–	–	–
B. subtilis	do	....	....	....	–	–	–	–
B. coli communis	Wilson method	+	+	–	–	–	–	–
B. subtilis	do	....	....	....	+	+	+	–

EXPERIMENT NO. 14b.

After the room in experiment No. 14 had been left closed 24 hours.

Quantity of formaldehyde found per cubic foot, 0.01028 gram.
Yield of formaldehyde, 9.6 per cent.
Weather: Gentle winds during intervening 24 hours.
Temperature of room during experiment, 84° F.
Relative humidity, 76 per cent.
Absolute humidity, 9.38 grams per cubic foot.

[+ means growth; − no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		5	10	25	30	45	60	120
B. pyocyaneus	Filter paper	+	+	−	−	−	−	−
B. coli communis	do	+	−	−	−	−	−	−
B. dysenteriæ	do	+	−	−	−	−	−	−
B. subtilis	do	+	+	+	+	+	−	−
B. coli communis	Wilson method	+	+	+	....	−	−	−
B. subtilis	do	+	+	+	+	+	+	−

EXPERIMENT NO. 15.

Dilute formalin-permanganate method.

Formalin, 600 c. c.
Water, 600 c. c.
Permanganate, 450 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 grams.
Quantity of formaldehyde found per cubic foot, 0.0279 gram.

(Determination made by drawing air from the room, beginning 15 minutes after mixing the formalin and permanganate, and consuming 1 hour 20 minutes in the drawing.)

Yield of formaldehyde, 26.1 per cent.
Weather: Light east wind, clear.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before mixing HCHO and $KMnO_4$	79.5	62	6.67
After 10 minutes	80.5	87	9.66
After 2 hours	81.0	81	9.13

See Note, Experiment 9.

[+ means growth; − no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		5	10	20	30	45	60	120
B. coli communis	Glass	−	−	−	−	−	−	−
B. subtilis	do	+	+	+	+	+	+	+
B. pyocyaneus	Filter paper	+	−	−	−	−	−	−
B. typhosus	do	+	−	−	−	−	−	−
B. subtilis	do	+	+	+	+	+	+	+
B. coli communis	Wilson method	+	+	−	−	−	−	−

Although the relative humidity in the above experiment was 62 per cent and the temperature 79.5° F., the spores of *B. subtilis* were not killed within two hours; this was due in all probability to the humidity being insufficient. The other organisms were killed within a few minutes. The humidity was increased 25 per cent by the moisture given off, but this did not seem to answer the purpose in case of the spores.

The following two experiments (Nos. 16 and 17) were done during colder weather than the foregoing. The results show a marked decrease in the percentage of formaldehyde obtained, as well as diminished germicidal powers. About the same hazy appearance in the room was observed as with the undiluted formalin-permanganate method under similar conditions of temperature, but the deposit of paraform was not so marked.

Comparing experiments Nos. 7 and 8 (undiluted formalin-permanganate) with Nos. 16 and 17 it will be observed that, although the temperature was lower in the latter experiments than in the former, the germicidal results are in favor of the latter experiments.

EXPERIMENT NO. 16.

Dilute formalin-permanganate method.

Formalin, 600 c. c.
Water, 300 c. c.
Permanganate, 375 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.01626 gram.

(Determination made by drawing air from the room, beginning 15 minutes after mixing the formalin and permanganate, and consuming 1 hour 5 minutes in the drawing.)

Yield of formaldehyde, 15.2 per cent.
Weather: Light clouds, northwest breeze.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before mixing HCHO and $KMnO_4$	39	51	1.39
After 10 minutes	42	92	2.82
After 3 hours	40	83	2.36

See Note, Experiment No. 9.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.							
		10	20	30	45	60	90	120	180
B. coli communis	Filter paper	+	–	–	–	–	–	–	–
B. typhosus	do	+	–	–	–	–	–	–	–
B. subtilis	do	+	+	+	+	+	+	+	+
B. coli communis	Wilson method	+	+	+	+	+	+	+	–
B. subtilis	do	+	+	+	+	+	+	+	+

EXPERIMENT NO. 17.

Dilute formalin-permanganate method.

Formalin, 600 c. c.
Water, 600 c. c.
Permanganate, 450 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.01234 gram.
(Determination made by drawing air from the room, beginning 15 minutes after mixing the formalin and permanganate, and consuming 1 hour 30 minutes in the drawing.)
Yield of formaldehyde, 11.53 per cent.
Weather: Cloudy, north wind.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before mixing HCHO and $KMnO_4$	41	55	1.62
After 10 minutes	40	92	2.51
After 3 hours	41.5	61	1.83

See Note, Experiment No. 9.

[+ means growth; − no growth.]

Organism.	How exposed.	Time of exposure in minutes, and result.							
		10	20	30	45	60	90	120	180
B. coli communis	Filter paper	+	−	−	−	−	−	−	−
B. typhosus	do	+	−	−	−	−	−	−	−
B. subtilis	do	+	+	+	+	+	+	+	+
B. coli communis	Wilson method	+	+	+	+	+	+	+	+
B. subtilis	do	+	+	+	+	+	+	+	+

For practical disinfection purposes the results of the undiluted and diluted formalin-permanganate methods do not, on the whole, differ materially. The relative humidity is increased in about the same proportions in the two methods and the organisms killed in about the same time. The only advantage in diluting the formalin seems to be when working at a reduced temperature. Then polymerization does not seem to take place as much as when the water is not added. This may account for the relatively larger percentage yield of formaldehyde, as well as its more efficient germicidal action in experiments Nos. 16 and 17 as compared with experiments Nos. 7 and 8.

Comparing experiment No. 16, in which 300 c. c. of water were added, with experiment No. 17, in which 600 c. c. of water were added, it will be seen that the addition of the former quantity of water gave slightly better germicidal results than the latter. This would indicate that there is nothing to be gained by diluting the quantity of formalin with more than half its volume of water. This statement, however, must not be construed as recommending the diluted formalin-permanganate method for disinfection purposes during cold weather, but simply as showing that under these conditions it possesses a slight advantage over the undiluted formalin-permanganate method.

Except under the conditions as stated, the addition of the water is a disadvantage, as the percentage of formaldehyde liberated is diminished in proportion to the quantity of water used, and, furthermore, a larger quantity of permanganate is required.

III. RETORT WITHOUT PRESSURE.[a]

The formalin in this method is simply heated to the boiling point under atmospheric pressure in a retort, and the issuing water vapor and formaldehyde gas are passed into the room through a suitable tube.

In the following experiments 600 c. c. of formalin, to which were added 6 c. c. of glycerine, were taken for the charge, and the heat was continued under the retort until vapor practically ceased to issue from the outlet tube.

When the retort was removed, there was practically no liquid left in it. The time required to vaporize the formalin varied from 1 hour to 1 hour and 10 minutes in the different experiments, depending upon the degree of the temperature.

Chemical determinations.—These determinations were made by drawing air from the room, beginning usually a short time after exhausting the retort; however, two were made after waiting 3 hours and 40 minutes, and 72 hours, respectively. The method of procedure has been described, page 11.

The results are shown in the following table:

TABLE 3.—*Experiments with the retort without pressure.*

Number.	Temperature of room before experiment.	Time required to exhaust retort.	Time between removal of retort and drawing air.	Time required to draw air.	Volume of air drawn, in liters.	Relative humidity before experiment.	Relative humidity at end of injection.	Increase in grains of moisture per cubic foot.	Abs. formaldehyde used per cubic foot.	Abs. formaldehyde found per cubic foot.	Per cent yield.	Condition of wind.
	°*F.*	*h. m.*	*h. m.*	*h. m.*					*gm.*	*gm.*		
I	73	1 0	0 22	1 6	10	73	86	1.5	0.107	0.05037	47.07	Very slight breeze.
IIa	76	1 0	0 21	1 10	10	82	89	0.96	.107	.04935	46.12	Do.
IIb			3 40	1 4	8	......	......	......	.107	.04183	39.1	Do.
IIc			72 0	1 7	10	......	......	......	.107	.00637	5.95	Strong wind July 29, 30, 31.
III	40	1 10	0 15	1 20	12	52	69	.67	.107	.01982	18.4	Light wind.

---

[a] The retort used is the Trenner Lee formaldehyde disinfector, manufactured by J. Ellwood Lee Company, Conshohocken, Pa.

With comparatively high temperatures, say, 75° F., the yield of formaldehyde gas by this method was appreciably higher than by the autoclave method, although the time required to exhaust the apparatus was more than twice as long in the former than in the latter. Because of this longer time there was naturally more loss by leakage in the retort method before beginning to draw air. The higher percentage would be expected, since in the retort the formaldehyde is not subjected to so high a temperature and pressure as in the autoclave.

Experiment II *c* shows the loss by leakage after the room in experiment II had been left closed 72 hours. Although the yield was greater in the retort than by any other method tried, there arises the question, What becomes of the rest of the formaldehyde? This point is an interesting one and worthy of further investigation, but will not be taken up in this work except in so far as a reduction in temperature affects the percentage of formaldehyde obtained. This reduction is shown in experiment III (temperature 40° F.,) in which only 18.4 per cent was obtained as against 46.12 per cent in experiment II (temperature 73° F.)

A slightly lower temperature with this method seems necessary to produce the same appearance in the room that is observed in the undiluted formalin-permanganate method; that is, about 55° F. with this method as against 60° to 65° F. with the method referred to. Even then the deposit of paraform is not so marked as with the undiluted formalin-permanganate method. The reason for this is not apparent, since the percentages obtained with the two methods showed about the same relative decrease.

The quantity of paraform deposited in the room is about the same with this method as with the diluted formalin-permanganate and autoclave methods.

Germicidal properties.—As it took approximately 1 hour to exhaust the quantity of formalin used in these experiments, the organisms were not exposed to the action of the formaldehyde until this process was completed. The organisms were prepared and exposed in the usual manner previously described.

A summary of conditions and results is given in each of the following experiments:

EXPERIMENT NO. 18.

Retort without pressure.

Formalin, 600 c. c.; glycerin, 6 c. c.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Time required to exhaust material in retort, 1 hour.
Quantity of formaldehyde found per cubic foot, 0.05037 gram.
(Determination made by drawing air from room, beginning 22 minutes after exhausting retort and consuming 1 hour 6 minutes in the drawing.)
Yield of formaldehyde, 47.07 per cent.
Weather: Slight wind southwest, cloudy.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before beginning to introduce the gas	73.5	73	6.40
After 1 hour	74.5	86	7.91
After 2 hours	75.0	86	8.00

NOTE.—In all the experiments with this method the temperature and humidity are given from the time of beginning to introduce the gas. The organisms were exposed immediately after exhausting the retort.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		2	5	10	20	30	60
B. pyocyaneus	Filter paper	+	+	–	–	–	–
B. coli communis	do	+	+	–	–	–	–
B. dysenteriæ	do	+	+	–	–	–	–
B. subtilis	do	+	+	+	+	+	–
B. coli communis	Wilson method	+	+	+	–	–	–
B. subtilis	do	+	+	+	+	+	–

EXPERIMENT NO. 19.

Retort without pressure.

Formalin, 600 c. c.; glycerin, 6 c. c.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Time required to exhaust material in retort, 1 hour.
Percentage yield of formaldehyde undetermined.
Weather: Slight westerly wind, clear.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before beginning to introduce the gas	74.5	60	5.52
After 1 hour	75.0	84	7.85
After 2 hours	75.0	82	7.66

See note, Experiment No. 18.

EXPERIMENT NO. 19—Continued.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		2	5	10	20	30	60
B. pyocyaneus	Filter paper	+	+	–	–	–	–
B. coli communis	do	+	+	–	–	–	–
B. dysenteriæ	do	+	–	–	–	–	–
B. subtilis	do	+	+	+	–	–	–
B. coli communis	Wilson method	+	+	+	+	–	–
B. subtilis	do	+	+	+	+	+	–

EXPERIMENT NO. 20.

Retort without pressure.

Formalin, 600 c. c.; glycerin, 6 c. c.

Capacity of room, 2,000 cubic feet.

Quantity of formaldehyde used per cubic foot, 0.107 gram.

Time required to exhaust material in retort, 1 hour.

Quantity of formaldehyde found per cubic foot, 0.04708 gram.

(Determination made by drawing air from room beginning 25 minutes after exhausting retor and consuming 1 hour 20 minutes in the drawing.)

Yield of formaldehyde, 44 per cent.

Weather: Rather strong northwest wind; clear.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before beginning to introduce the gas	74	74	6.70
After 1 hour	75	84	7.85
After 2 hours	74	89	8.06

See Note, experiment No. 18.

[+ means growth; – no growth].

Organism.	How exposed.	Time of exposure in minutes, and results.					
		5	10	20	30	45	60
B. pyocyaneus	Filter paper	–	–	–	–	–	–
B. coli communis	do	+	–	–	–	–	–
B. dysenteriæ	do	–	–	–	–	–	–
B. subtilis	do	+	+	0	–	–	–
B. coli communis	Wilson method	+	+	+	–	–	–
B. subtilis	do	+	+	+	+	–	–

EXPERIMENT NO. 21.

Retort without pressure.

Formalin, 600 c. c.; glycerin, 6 c. c.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Time required to exhaust material in retort, 1 hour.
Quantity of formaldehyde found per cubic foot, 0.04935 gram.

(Determination made by drawing air from room, beginning 21 minutes after exhausting retort and consuming 1 hour 10 minutes in the drawing.)

Yield of formaldehyde, 46.12 per cent.
Weather: Light westerly wind; cloudy.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before beginning to introduce the gas	76	82	7.91
After 1 hour	77	89	8.87
After 2 hours	77	89	8.87

See Note, experiment No. 18.

[+ means growth; — no growth].

Organism.	How exposed.	Time of exposure in minutes, and results.				
		2	5	10	25	60
B. pyocyaneus	Filter paper	+	—	—	—	—
B. coli communis	.....do	+	—	—	—	—
B. dysenteriæ	.....do	+	—	—	—	—
B. subtilis	.....do	+	+	+	—	—
B. coli communis	Wilson method	+	+	—	—	—
B. subtilis	.....do	+	+	+	+	—

For surface disinfection better results could not be desired than were obtained in the above experiments. The temperature and humidity being comparatively high, the nonspore-bearing organisms were killed within five to ten minutes and the spores of *B. subtilis* within one hour, the time varying slightly in the different experiments.

Although the humidity in experiment No. 19 was only 60 per cent before starting, the results are about as good as in those in which the humidity was considerably higher.

In the following experiment (No. 21ᵃ) which was done after the room in experiment No. 21 had been left closed 72 hours (3 days), only 5.95 per cent of formaldehyde was found. Still *B. pyocyaneus* and *B. coli communis* exposed on filter paper were killed within 1 hour and *B. dysenteriæ* within 30 minutes. This shows, as has been previously mentioned, what a small percentage of formaldehyde will kill nonspore-bearing organisms when we have proper temperature and humidity. The spores of *B. subtilis* were not killed within 2 hours.

EXPERIMENT NO. 21a.

After the room in experiment No. 21 had been left closed 72 hours (3 days).

Quantity of formaldehyde found per cubic foot, 0.00637 gram.
Yield of formaldehyde, 5.95 per cent.
Weather: Rather strong wind during the 3 days; rained several times.
Temperature of room during experiment, 75° F.
Relative humidity, 78 per cent.
Absolute humidity, 7.30 grams per cubic foot.

[+ means growth; — no growth].

Organism.	How exposed.	Time of exposure in minutes, and results.					
		5	10	20	30	60	120
B. pyocyaneus	Filter paper	+	+	+	+	−	−
B. coli communis	do	+	+	+	+	−	−
B dysenteriæ	do	+	+	+	−	−	−
B. subtilis	do	+	+	+	+	+	+
B. coli communis	Wilson method	+	+	+	+	+	+
B. subtilis	do	+	+	+	+	+	+

The following experiment (No. 22) was done at a comparatively low temperature, in consequence of which the percentage of formaldehyde gas obtained was small. The same hazy appearance of the air in the room was observed as with the previous methods under similar conditions of temperature. The deposit of paraform was not so marked as with the undiluted formalin-permanganate method, but about the same as with the diluted method.

Considering the difference in temperature, the germicidal action of the formaldehyde in this experiment is better than in experiment No. 8 with the formalin-permanganate method.

EXPERIMENT NO. 22.

Retort without pressure

Formalin, 600 c. c.; glycerin, 6 c. c.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Time required to exhaust material in retort, 1 hour and 10 minutes..
Quantity of formaldehyde found per cubic foot, 0.01982 gram.
(Determination made by drawing air from room, beginning 15 minutes after exhausting retort and consuming 1 hour and 20 minutes in the drawing).
Yield of formaldehyde. 18.4 per cent.
Air in room had a very hazy appearance during the experiment, probably due to polymerization of formaldehyde.
Weather: Clear and cold; light northeast wind.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	Per cent.	
Before beginning to introduce the gas	40	52	1.47
After 1 hour	42	69	2.11

See Note, Experiment No. 18.

EXPERIMENT NO. 22—Continued.

[+ means growth; — no growth].

Organism.	How exposed.	Time of exposure in minutes, and results.						
		10	20	30	45	60	120	180
B. typhosus	Filter paper	+	+	+	—	—	—	—
B. coli communis	do	+	+	+	+	—	—	—
B. subtilis	do	+	+	+	+	+	+	+
B. coli communis	Wilson method	+	+	+	+	+	+	—
B. subtilis	do	+	+	+	+	+	+	+

What has been said in regard to temperature, humidity, and percentage of formaldehyde with the formalin-permanganate method practically applies to this method also. This method might be employed when the temperature is a few degrees lower than is permissible with the formalin-permanganate method; but the difference is not sufficient to be of much practical importance. Apparently polymerization does not take place quite so markedly, but about the same comparative reductions in the percentage of formaldehyde obtained are observed with the two methods when the temperature is low.

The time required to destroy microorganic life is about the same in the two methods. The principal objections to the retort method are that special apparatus is required and considerable time is consumed in vaporizing the formalin.

IV. THE AUTOCLAVE UNDER PRESSURE.[a]

Six hundred c. c. of formalin, 60 c. c. of glycerin, 120 gm. of calcium chlorid, and water sufficient to make a volume of 1,000 c. c. constituted the charge for each experiment. The pressure was raised to between 50 and 60 pounds, when the cock was opened and injection continued till the pressure had fallen to about 40 pounds. Then the pressure was raised and vapor again injected.

This process was repeated until the pressure arose very slowly and vapor ceased to escape from the nozzle of the autoclave, which was inserted through a suitable hole in the door. After each experiment there was very little liquid left in the autoclave.

The time required to vaporize the quantity of formalin, etc., used varied from 22 to 28 minutes in the different experiments, depending upon the temperature of the atmosphere.

Chemical determinations.—The method of procedure has been dedescribed.

The results are shown in the following table:

---

[a] The one used is the Kinyoun-Francis autoclave, manufactured by the Kensington Engine Works (Limited), Philadelphia, Pa.

TABLE 4.—*Experiments with the autoclave.*

Number.	Temperature of room before experiment.	Time required to exhaust autoclave.	Time between beginning of discharge to drawing of air.	Time required to draw air.	Volume of air drawn, in liters.	Relative humidity before experiment.	Relative humidity 5 minutes after removing autoclave.	Increase in grains of moisture per cubic foot.	Abs. formaldehyde used per cubic foot.	Abs. formaldehyde found per cubic foot.	Per cent yield.	Weather conditions.
	° F.	*m.*	*h. m.*	*h. m.*					*gm.*	*gm.*		
I	80	25	3 32	1 18	10	75	96	2.96	0.107	0.0406	37.94	Almost no wind.
II	31.5	25	*a*0 35	1 17	10	81	94	2.00	.107	0.04213	39.37	Good breeze.
			*b*3 33	1 4	10	......	......	......	.107	0.0216	20.18	Storm.
III	80	22	*a* 40	1 10	11	75	94	2.23	.107	0.04448	41.57	Almost no wind.
			*b*1 41	1 20	11	......	......	......	.107	0.04116	38.46	Do.
IV	49	25	0 15	1 7	10	67	81	.78	.107	0.1851	17.30	No wind.
V	41	23	0 15	1 30	10	69	92	.77	.107	0.01471	13.75	Light breeze.

From the result of III *a* it seems probable that on a warm calm day in a fairly tight room the amount of formaldehyde by the autoclave from 600 c. c. of formalin would be approximately 42 per cent after an interval of about 15 minutes from the time of removing the nozzle from the room.

II *a* is lower than III *a*, which was to be expected, because of the strong wind.

II *b* shows the rapid loss of formaldehyde gas during a strong wind or storm. During the time that the air was drawn in this experiment a severe windstorm was in progress, with the result that during the interval of 3 hours between *a* and *b* nearly half the formaldehyde escaped from the room.

In these, as in the determinations in the case of the other methods of charging the room, the results represent the average quantity of formaldehyde for the interval during which the air was drawn.

Even during warm weather (80° F.) apparently over half of the formaldehyde gas in the 600 c. c. of formalin used to charge the room was lost. It is altogether improbable that the most of this escaped from the room during the 25 minutes required to exhaust the autoclave, as is evident from the slow rate of loss on a calm day shown in I and III *a* and *b*. It is more probable that under the high temperature and pressure in the autoclave the formaldehyde undergoes change. The effects of a reduced temperature upon formaldehyde liberated by this method were about the same as those observed in case of the other methods already described. The reduction in percentage of formaldehyde obtained is shown in experiments IV, temperature 49° F., and V, temperature 41° F., which gave 17.30 per cent and 13.75 per cent, respectively.

Germicidal properties.—The method of procedure has been described, the organisms being exposed after all the formalin, etc., had been vaporized and injected.

The following are the conditions and results of the different experiments.

EXPERIMENT NO. 23.

Autoclave.

Formalin, 600 c. c.; glycerin, 60 c. c.; calcium chloride, 120 grams; water sufficient to make 1,000 c. c.

Capacity of room, 2,000 cubic feet.

Quantity of formaldehyde used per cubic foot, 0.107 gram.

Time required to exhaust material in autoclave, 25 minutes.

Quantity formaldehyde found per cubic foot, 0.04213 gram.

(Determination made by drawing air from room beginning 35 minutes after exhausting autoclave and consuming 1 hour 17 minutes in the drawing.)

Yield of formaldehyde, 39.37 per cent.

Weather: Moderate westerly wind; cloudy.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before beginning to introduce the gas	81.5	81	9.26
After 30 minutes	83.0	94	11.26
After 2 hours	82.0	86	10.00

NOTE.—In all the experiments with this method the temperature and humidity are given from the time of beginning to introduce the gas; the organisms were exposed immediately after exhausting the autoclave.

[+ means growth; — no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		2	5	10	20	30	60
B. coli communis	Glass	+	—	—	—	—	—
B. pyocyaneus	Filter paper	+	+	—	—	—	—
B. typhosus	do	+	—	—	—	—	—
B. coli communis	do	+	+	—	—	—	—
B. dysenteriæ	do	+	+	—	—	—	—
B. subtilis	do	....	....	—	—	—	—
B. coli communis	Wilson method	+	+	—	—	—	—

EXPERIMENT NO. 24.

Autoclave.

Formalin, 600 c. c.; glycerin, 60 c. c.; calcium chloride, 120 grams; water sufficient to make 1,000 c. c.

Capacity of room, 2,000 cubic feet.

Quantity of formaldehyde used per cubic foot, 0.107 gram.

Time required to exhaust material in autoclave, 25 minutes.

Quantity formaldehyde found per cubic foot, 0.0406 gram.

(Determination made by drawing air from room beginning 3 hours 32 minutes after exhausting autoclave and consuming 1 hour 18 minutes in the drawing.)

Yield of formaldehyde, 37.94 per cent.

Weather: Practically no wind; cloudy.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before beginning to introduce the gas	80	75	8.20
After 30 minutes	82	96	11.16
After 2 hours	81	91	10.58

See Note, Experiment No. 23.

EXPERIMENT NO. 24—Continued.

[+ means growth; − no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		2	5	10	20	30	60
B. coli communis	Glass	−	−	−	−	−	−
B. subtilis	do	....	....	....	−	−	−
B. pyocyaneus	Filter paper	+	−	−	−	−	−
B. typhosus	do	+	−	−	−	−	−
B. coli communis	do	+	−	−	−	−	−
B. coli communis	Wilson method	+	+	−	−	−	−
B. subtilis	do	....	....	....	−	−	−

EXPERIMENT NO. 25.

Autoclave.

Formalin, 600 c. c.; glycerin, 60 c. c.; calcium chloride, 120 grams; water sufficient to make 1,000 c. c.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Time required to exhaust material in autoclave, 22 minutes.
Quantity formaldehyde found per cubic foot, 0.04448 gram.
(Determination made by drawing air from room, beginning 40 minutes after exhausting autoclave, and consuming 1 hour 10 minutes in the drawing.)
Yield of formaldehyde, 41.57 per cent.
Weather: Slight westerly breeze.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before beginning to introduce the gas	80.0	75	8.20
After 30 minutes	80.5	94	10.43
After 2 hours	81.0	94	10.59

See Note, experiment No. 23.

[+ means growth; − no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		2	5	10	20	30	60
B. pyocyaneus	Filter paper	+	+	−	−	−	−
B. coli communis	do	+	−	−	−	−	−
B. dysenteriæ	do	+	+	−	−	−	−
B. subtilis	do	+	+	+	−	−	−
B. coli communis	Wilson method	+	+	+	−	−	−
B. subtilis	do	+	+	+	+	+	−

The above experiments show very efficient germicidal results. Even the spores of *B. subtilis* exposed by the Wilson method were killed within 10 minutes in experiments Nos. 23 and 24.

In experiment No. 25 the time required to kill the different organisms was prolonged as compared with the two previous experiments;

this was especially so with the spores of *B. subtilis* exposed by the Wilson method. The reason for this is not apparent, since the temperature and humidity were practically the same in the different experiments.

In the following experiment (No. 26) the germicidal power of the formaldehyde was much diminished as a result of the low humidity—46 per cent—before starting the experiment.

EXPERIMENT NO. 26.

Autoclave.

Formalin, 600 c. c.; glycerin, 60 c. c.; calcium chloride, 120 grams; water sufficient to make 1,000 c. c.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Time required to exhaust material in autoclave, 22 minutes.
Yield of formaluehyde, undetermined.
Weather: Rather strong westerly wind.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before beginning to introduce the gas	68	46	3.45
After 30 minutes	69	67	5.17
After 2 hours	69	60	4.63

See Note, experiment No. 23.

[+ means growth; − no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		5	15	30	45	60	120	180
B. pyocyaneus	Glass	+	+	+	+	−	−	....
B typhosus	....do	+	+	+	−	−	−	....
B. staph. py. aureus	....do	+	+	+	+	−	−	....
B. coli communis	....do	+	+	+	+	−	−	....
B. pyocyaneus	Filter paper	+	+	+	+	+	−	....
B. coli communis	Wilson method	+	+	+	+	+	+	....

When working at reduced temperatures the same decrease in percentage yield of formaldehyde and the same deficiency in germicidal action are observed in this method as in the formalin-permanganate and the retort methods. This is shown in the following two experiments, in which the spores of *B. subtilis* were not usually killed within 3 hours, although the nonspore-bearing organisms exposed directly on slips of filter paper were killed in a fairly short time.

Comparing the results of these two experiments with experiment No. 26, it will be seen that a reduction in humidity diminishes the killing power of formaldehyde more than does a similar reduction in temperature.

EXPERIMENT NO. 27.

Autoclave.

Formalin, 600 c. c.; glycerin, 60 c. c.; calcium chloride, 120 grams; water sufficient to make 1,000 c. c.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Time required to exhaust material in autoclave, 25 minutes.
Quantity formaldehyde found per cubic foot, 0.01851 gram.

(Determination made by drawing air from room, beginning 15 minutes after exhausting autoclave, and consuming 1 hour 7 minutes in the drawing.)

Yield of formaldehyde, 17.30 per cent.
Weather: Very little wind.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before beginning to introduce the gas	49	67	2.63
After 30 minutes	51	81	3.41
After 2 hours	54	61	2.85

See Note, experiment No. 23.

[+ means growth; — no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		10	20	30	45	60	120	180
B. coli communis	Filter paper	+	—	—	—	—	—	—
B. typhosus	do	—	—	—	—	—	—	—
B. subtilis	do	+	+	+	+	+	—	—
B. coli communis	Wilson method	+	+	+	+	+	—	—
B. subtilis	do	+	+	+	+	+	+	+

EXPERIMENT NO. 27.

Autoclave.

Formalin 600 c. c., glycerin 60 c. c., calcium chloride 120 grams, water sufficient to make 1,000 c. c.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Time required to exhaust material in autoclave, 28 minutes.
Quantity formaldehyde found per cubic foot, 0.01471 gram.

(Determination made by drawing air from room, beginning 15 minutes after exhausting autoclave and consuming 1 hour 30 minutes in the drawing.)

Yield of formaldehyde, 13.75 per cent.
Weather: Damp and snow on ground; light wind.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before beginning to introduce the gas	41	69	2.04
After 30 minutes	42	92	2.81
After 2 hours	42	84	2.57

See Note, Experiment No. 23.

EXPERIMENT NO. 27—Continued.

[+ means growth, — no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		10	20	30	45	60	120	180
B. coli communis	Filter paper	+	+	—	—	—	—	—
B. typhosus	do	+	—	—	—	—	—	—
B. subtilis	do	+	+	+	+	+	+	+
B. coli communis	Wilson method	+	+	+	+	+	—	—
B. subtilis	do	+	+	+	+	+	+	+

These results agree rather closely with those of the formalin-permanganate and retort methods and especially with the latter method, except that the retort liberates a slightly larger percentage of formaldehyde than does the autoclave. For disinfecting purposes the three methods give about the same results, provided the temperature and humidity are high. A low temperature affects the autoclave method in about the same way that it does the retort method. The disadvantages of the autoclave are that more or less complicated apparatus is required which is cumbersome to handle and that considerable time is consumed in vaporizing the formalin, although not half so long as with the retort.

V. SHEET-SPRAYING METHOD.

Two sheets, about 4½ by 6½ feet, were hung up in the room in a slanting position at an angle of about 45°. It was found advantageous to have them just damp to the touch when hung up, as the formalin was absorbed by the fibers more quickly and had very little tendency to run off the sheet. Six hundred c. c. of formalin were sprayed uniformly and the door closed.

Chemical determinations.—Different intervals were allowed to elapse after spraying the sheets before beginning to draw air from the room. The method of procedure has been described. The results are given in the following table:

TABLE 5.—*Experiments with the sheet-spraying method.*

Number.	Temperature of room before experiment.	Time between closing room and drawing air.		Time required to draw air.		Volume of air drawn, in liters.	Relative humidity before experiment.	Relative humidity after closing room.	Gain in grains of moisture per cubic foot.	Abs. formaldehyde used per cubic foot.	Abs. formaldehyde found per cubic foot.	Per cent.	Condition of wind
	° *F.*	*h.*	*m.*	*h.*	*m.*					*Gm.*	*Gm.*		
I*a*	76.5	1	26	1	13	10	72	[a] 83	1.6	0.107	0.02576	24.07	No wind.
I*b*		3	47	1	8	10	......	......	......	.107	.03262	30.48	Do.
II*a*	78	1	40	1	15	10	3	[b] 85	......	.107	.0252	23.55	Gentle breeze.
II*b*		4	5	1	7	10	......	......	......	.107	.0305	28.50	Do.
II*c*		23	30	1	17	10	......	......	......	.107	.02225	20.80	No wind.
III	43.5	1	30	1	25	11	52	61	0.05	.107	.00345	3.16	Gentle breeze.

[a] After 5 hours.

[b] After 3 hours.

Compared with the previous methods this one produces a fairly large quantity of formaldehyde gas in the air of the room when used during warm weather, say at a temperature of 75° F The results under these conditions show that the quantity increases during a relatively long period of time. In experiment II *c*, Table 5, the quantity of formaldehyde gas found after an interval of 22 hours was only 2.75 per cent less than that found in II *a*, 90 minutes after closing the room. During cold weather, say temperature of 43.5° F., very little formaldehyde is liberated, as the result of experiment III shows. Evaporation takes place very slowly and the formalin polymerizes on the sheets.

Germicidal properties.—In these experiments the organisms were prepared and exposed in the usual way, but not until one hour after spraying. This allowed time for at least a portion of the formaldehyde to be given off and become diffused.

A summary of conditions and results is given in each of the following experiments:

EXPERIMENT NO. 28.

Sheet spraying.

Formalin, 600 c. c.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.02576 gram.
(Determinations made by drawing air from room beginning 1 hour 26 minutes after spraying sheets and consuming 1 hour 15 minutes in the drawing.)
Yield of formaldehyde, 24.07 per cent.
Condition of weather: Very light wind, slightly cloudy.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before spraying	76.5	72	7.05
1 hour after	76.0	80	7.72
2 hours after	77.0	81	8.06

Organisms not exposed until 1 hour after spraying sheet.

[+ means growth; — no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		5	10	20	30	60	90	120
B. pyocyaneus	Filter paper	+	+	+	—	—	—	—
B. coli communis	do	+	+	—	—	—	—	—
Do	Wilson method	+	+	+	—	—	—	—
B. dysenteriæ	Filter paper	+	+	—	—	—	—	—
B. subtilis	do	+	+	+	+	—	—	—
Do	Wilson method	+	+	+	+	+	—	—

EXPERIMENT NO. 29.

Sheet spraying.

Formalin, 600 c. c.

Capacity of room, 2,000 cubic feet.

Percentage of formaldehyde liberated was not determined.

Condition of weather: Light northwest wind, clear.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before spraying	74.0	80	7.25
1 hour after	74.5	86	7.91
2 hours after	76.0	89	8.6

Organisms not exposed until 1 hour after spraying sheets.

[+ means growth; — no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		5	10	20	30	60	90	120
B. pyocyaneus	Filter paper	....	+	+	—	—	—	—
B. coli communis	do	....	+	—	—	—	—	—
Do	Wilson method	....	+	+	+	—	—	—
B. dysenteriæ	Filter paper	....	+	—	—	—	—	—
B. subtilis	do	....	+	+	+	—	—	—
Do	Wilson method	....	+	+	+	+	+	—

EXPERIMENT NO. 30.

Sheet spraying.

Formalin, 600 c. c.

Capacity of room, 2,000 cubic feet.

Quantity of formaldehyde used per cubic foot, 0.107 gram.

Quantity of formaldehyde found per cubic foot, 0.0252 gram.

(Determinations made by drawing air from room beginning 1 hour 40 minutes after spraying sheets and consuming 1 hour 15 minutes in the drawing.)

Yield of formaldehyde, 23.55 per cent.

Condition of weather: Light southerly wind, light clouds.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before spraying	78	83	8.52
1 hour after	77	87	8.67
2 hours after	79	85	9.01

Organisms not exposed until 1 hour after spraying sheets.

[+ means growth; — no growth.

Organism.	How exposed.	Time of exposure in minutes and results.						
		5	10	20	30	60	90	120
B. pyocyaneus	Filter paper	....	—	—	—	—	—	—
B. coli communis	do	....	—	—	—	—	—	—
Do	Wilson method	....	+	+	—	—	—	—
B. dysenteriæ	Filter paper	....	—	—	—	—	—	—
B. subtilis	do	....	—	—	—	—	—	—
Do	Wilson method	....	+	+	+	+	—	—
B. dysenteriæ	do	....	+	—	—	—	—	—

EXPERIMENT NO. 30 *a*.

After the room in experiment No. 30 had been left closed 24 hours.

Quantity of formaldehyde found per cubic foot, 0.02225 gram.
Yield of formaldehyde, 20.80 per cent.
Weather: No wind.
Temperature, 72° F.
Relative humidity, 91 per cent.
Absolute humidity, 8.51 per cubic feet.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		5	10	20	30	60	90	120
B. pyocyaneus	Filter paper	....	–	–	–	–	–	–
B. coli communis	....do	....	–	–	–	–	–	–
Do	Wilson method	....	+	–	–	–	–	–
B. dysenteriæ	Filter paper	....	–	–	–	–	–	–
B. subtilis	....do	....	....	....	–	–	–	–
Do	Wilson method	....	+	+	+	+	–	–

The exposed organisms were killed in the above experiments in a comparatively short time, but hardly as short as in the previous methods under similar conditions of temperature and humidity.

Experiment No. 30 *a* was tried after the room in experiment No. 30 had been left closed 24 hours. It shows that in a well-closed room like the one used by us the percentage of formaldehyde gas in the room was still comparatively high after 24 hours' standing and that the exposed organisms were killed then in about the same time that they were 1 hour after spraying the sheets.

The following experiment (No. 31) shows the inefficiency of this method for disinfection purposes during cold weather, temperature 43.5° F. In this experiment even the nonspore-bearing organisms were not killed within 3 hours and only 3.16 per cent of formaldehyde was found.

EXPERIMENT NO. 31.

Sheet spraying.

Formalin, 600 c. c.
Capacity of room, 2,000 cubic feet.
Quantity of formaldeyhde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.00345 gram.
(Determinations made by drawing air from room beginning 1 hour 30 minutes after spraying sheets and consuming 1 hour 15 minutes in the drawing.)
Yield of formaldehyde, 3.16 per cent.
Condition of weather: Clear and dry, light southwesterly breeze.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before spraying	43.5	52	1.67
1 hour after	43.0	55	1.74
3 hours after	50.0	61	2.48

Organisms not exposed until 1 hour after spraying sheets.

EXPERIMENT NO. 31—Continued.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		15	30	45	60	120	180
B. coli communis	Filter paper	+	+	+	+	+	+
B. typhosus	do	+	+	+	+	+	+
B. subtilis	do	+	+	+	+	+	+
B. coli communis	Wilson method	+	+	+	+	+	+
B. subtilis	do	+	+	+	+	+	+

This method is very reliable for surface disinfection during warm weather provided the sheets are properly sprayed. Not over 300 c. c. of formalin should be used for every 30 square feet of sheet surface. The percentage of formaldehyde remains comparatively high during a relatively long time. The maximum of 30 per cent seems to be reached after approximately 4 hours standing, but at the end of 24 hours, 20.8 per cent was still found.

This is quite different from the other methods in which the percentage of formaldehyde begins to diminish by leakage from the moment the charging is finished. Evaporation maintains the humidity at a fairly high percentage which, with the constancy of liberation of formaldehyde, makes the method very applicable when a large exposure is required. On the other hand the influence of a low temperature upon the results obtained with this method is more marked than with any other methods tried. It was found that at a temperature of 43.5° F. very little formaldehyde is liberated and that the formalin gradually polymerizes on the sheets. This of course makes the use of this method for disinfection purposes absolutely impracticable during cold weather.

VI. THE GENERATING LAMP.[a]

The lamp used in this work is one of the various lamps devised for the purpose of generating formaldehyde by oxidation of methyl alcohol. This is brought about by means of incandescent platinized asbestos, which has the power of causing the oxygen of the air and methyl alcohol vapor to combine according to the following reaction:

$$\underset{\text{methyl alcohol.}}{CH_4O} + O = \underset{\text{formaldehyde.}}{HCHO} + H_2O.$$

The platinized asbestos is heated by first burning some of the alcohol, after which the flame is extinguished. Alcohol continues to vaporize and the heat produced in the oxidation maintains the temperature of the apparatus. The time usually allowed for the exhaustion of the lamp is about 2 hours.

---

[a] The lamp used is manufactured by the Kuhn Formaldehyde Generator Co., Alexandria, Va.

One thousand c. c. of commercial methyl alcohol were placed in the reservoir of the lamp and 1,500 c. c. of water in the basin around the reservoir.

In a preliminary experiment it was found necessary to have two silver nitrate tubes to absorb the hydrocyanic acid which was carried over from the cyanide absorption tubes by the air bubbling through them. This was no doubt due to the presence of a relatively large amount of carbonic acid and perhaps a small quantity of formic acid.

While formaldehyde is formed by the action of the platinized asbestos in the lamp, it is more than likely that it is not the only product of oxidation, but that the oxidation extends as far as the formation of carbonic acid. It is known that formic acid is oxidized to carbonic acid by platinized asbestos. The presence of either carbonic or formic acid in the air bubbling through the cyanide absorption tubes would cause the liberation of some hydrocyanic acid, which would be carried over. The precipitate in the first silver nitrate tube was somewhat greater than in the case of the other methods. The precipitate in the second silver nitrate tube did not appear until toward the end of the experiment.

The chemical determinations were made as follows:

I. Began to draw air 2 hours after lamp was started; volume of air drawn, 10 liters; time required, 1 hour 13 minutes; condition of wind, gentle breeze.

Temperature of room before experiment, 79° F.; relative humidity, 87 per cent.

Temperature of room 2 hours after starting lamp, 85° F.; relative humidity, 98 per cent.

Formaldehyde found per cubic foot, 0.01096 gram.

II. Began to draw air 2 hours after starting lamp; volume of air drawn, 10 liters; time required, 1 hour 6 minutes; condition of wind, moderate breeze.

Temperature of room before experiment, 75° F.; relative humidity, 82 per cent.

Temperature of room 2 hours after starting lamp, 92° F.; relative humidity, 96 per cent.

Formaldehyde found per cubic foot, 0.00986 gram.

In another experiment, which was done during colder weather than the above, the following are the results:

III. Began to draw air 2 hours after starting lamp; volume of air drawn, 10 liters; time required, 1 hour 15 minutes; condition of wind; light breeze.

Temperature of room before experiment, 42° F.; relative humidity, 69 per cent.

Temperature of room 2 hours after starting lamp, 49° F.; relative humidity, 86 per cent.

Formaldehyde found per cubic foot, 0.00424 gram.

As the formaldehyde is derived from methyl alcohol it is impossible to make a comparison of the percentages of formaldehyde obtained by this method with those obtained by the other methods in which formalin is used instead of alcohol.

Germicidal properties.—In all but two of the experiments the organisms were placed in the wire trays in the room before the lamp was started and the time of exposure estimated from the moment of starting the lamp and closing the room. This appeared to be the most rational way of estimating the time of exposure, as the lamp was in the room and it was impossible to determine the time at which the process of evolving the gas was completed. The room was not closed until it was found that the formaldehyde gas was being given off by the lamp. This was detected by its irritating action on the mucous membrane of the eyes and nose. However, in two experiments the exposures were made 2 hours after starting the lamp and closing the room.

A summary of conditions and results is given in each of the following experiments:

EXPERIMENT NO. 32.

Generating lamp.

Methyl alcohol, 1,000 c. c.[a]
Capacity of room, 2,000 cubic feet.
Weather: No wind, slightly cloudy.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before starting lamp	73	82	7.20
After 1 hour	81	85	9.58
After 2 hours	81	98	11.05
After 3 hours	79	98	11.05

Time of exposure of organisms counted from time of starting the lamp.

[+ means growth; –, no growth.]

Organism.	How exposed.	Time of exposure in minutes and results.						
		10	20	30	45	60	120	180
B. coli communis	Glass	+	+	+	–	–	–	–
B. subtilis	....do	+	+	+	+	+	–	–
B. pyocyaneus	Filter paper	+	+	+	–	–	–	–
B. typhosus	....do	+	+	+	–	–	–	–
B. coli communis	....do	+	+	+	–	–	–	–
B. subtilis	....do	+	+	+	+	+	–	–
B. coli communis	Wilson method	+	+	+	+	+	–	–

---

[a] For a room of this size the United States Quarantine regulations require 1,400 c. c. of methyl alcohol.

EXPERIMENT NO. 33.

Generating lamp

* Methyl alcohol, 1,000 c. c.
Capacity of room, 2,000 cubic feet.
Weather: Light southwest wind, clear.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before starting lamp	79	87	9.23
After 1 hour	86	88	11.35
After 2 hours	85	98	12.48
After 3 hours	84	98	12.11

Time of exposure of organisms counted from time of starting the lamp.

[+means growth; — no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		10	20	30	60	90	120	180
B. pyocyaneus	Filter paper	+	+	+	–	–	–	–
B. coli communis	do	+	+	+	–	–	–	–
B. dysenteriæ	do	+	+	+	–	–	–	–
B. subtilis	do	+	+	+	+	+	–	–
B. coli communis	Wilson method	+	+	+	–	–	–	–
B. subtilis	do	+	+	+	+	+	+	+

EXPERIMENT NO. 34.

Generating lamp.

* Methyl alcohol, 1,000 c. c.
Capacity of room, 2,000 cubic feet.
Weather: No wind, cloudy.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before starting lamp	64	84	5.51
After 2 hours	69	94	7.26
After 3 hours	67	92	6.66

Time of exposure of organisms counted from time of starting the lamp.

EXPERIMENT NO. 34—Continued.

[+ means growth; — no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.			
		30	60	120	180
B. pyocyaneus	Glass	—	—	—	—
B. typhosus	do	—	—	—	—
B. coli communis	do	+	—	—	—
Staphylococcus pyog. aur	do	+	—	—	—
B. typhosus	Filter paper	—	—	—	—
B. pyocyaneus	Wilson method	+	—	—	—

Most of the nonspore-bearing organisms were killed within 1 hour in the above experiments. The spores of *B. subtilis* exposed directly on slips of filter paper were destroyed within 2 hours, while the same organisms exposed by the Wilson method were not killed within 3 hours.

In each experiment the relative humidity was raised almost to saturation at the end of 2 hours; the temperature was also raised several degrees.

The following three experiments (Nos. 35, 36, and 37) were done at a lower temperature than the above experiments. In these the temperature and humidity were raised very materially. Most of the nonspore-bearing organisms were killed within 2 or 3 hours.

In Nos. 36 and 37 the organisms were not exposed until 2 hours after starting the lamp.

EXPERIMENT NO. 35.

Generating lamp.

* Methyl alcohol, 1,000 c. c.
Capacity of room, 2,000 cubic feet.
Weather: Cloudy, light wind.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before starting lamp	42	69	2.11
After 2 hours	49	86	3.38
After 4 hours	52	84	3.66

Time of exposure of organisms counted from time of starting the lamp.

EXPERIMENT NO. 35—Continued.

[+ means growth; − no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.				
		30	60	120	180	240
B. typhosus	Filter paper	+	+	−	−	−
B. coli communis	.....do	+	+	−	−	−
B. subtilis	.....do	+	+	+	+	+
B. coli communis	Wilson method	+	+	+	−	−
B. subtilis	.....do	+	+	+	+	+

EXPERIMENT NO. 36.

Generating lamp.

* Methyl alcohol, 1,000 c. c.
Capacity of room, 2,000 cubic feet.
Weather: Moderate, northerly wind; clear.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before starting lamp	44	63	2.07
After 2 hours	52	94	4.11
After 5 hours	51	87	3.68

Organisms exposed 2 hours after starting lamp.

[+ means growth; − no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		10	20	30	45	60	120	180
B. typhosus	Filter paper	+	+	+	+	−	−	−
B. coli communis	.....do	+	+	+	+	−	−	−
B. subtilis	.....do	+	+	+	+	+	+	+
B. coli communis	Wilson method	+	+	+	+	+	+	−
B. subtilis	.....do	+	+	+	+	+	+	+

EXPERIMENT NO. 37.

Generating lamp.

* Methyl alcohol, 1,000 c. c.
Capacity of room, 2,000 cubic feet.
Weather: No wind; snowing.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before starting lamp	39	59	1.62
After 2 hours	45	93	3.17
After 5 hours	39	92	2.71

Organisms exposed 2 hours after starting lamp.

EXPERIMENT NO. 37—Continued.

[+ means growth; − no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.						
		10	20	30	45	60	120	180
B. typhosus	Filter paper	+	+	+	+	+	−	−
B. coli communis	do	+	+	+	+	+	+	+
B. subtilis	do	+	+	+	+	+	+	+
B. coli communis	Wilson method	+	+	+	+	+	+	+
B. subtilis	do	+	+	+	+	+	+	+

* For rooms of this size the U. S. Quarantine regulations require 1440 c. c. of methyl alcohol.

With this method, under proper conditions of temperature and humidity, most of the nonspore-bearing organisms were killed within a reasonable time. The time, however, is longer than with the previous methods. Spores usually escaped destruction or were killed only after a relatively long time.

As has been stated, the organisms were usually exposed and the time of exposure counted from the time of starting the lamp. This was not the case with the retort and autoclave methods, which required approximately 1 hour for the former and 25 minutes for the latter to vaporize the formalin. Therefore, in making a comparison of the time required for germicidal efficiency in these three methods the time at which the exposures were made must be borne in mind.

With the lamp the organisms were, of course, subjected to the action of the formaldehyde as it was given off and probably many of them were destroyed before the process of liberation was complete. This fact also is to be considered in comparing the time actually required to kill the organisms in the three methods.

The lamp is the only method that raises the temperature to any appreciable extent. The humidity is also increased considerably, and as it comes principally from evaporation of the water in the lamp, it is very probable that it is more like the natural humidity of the atmosphere, and consequently is more potent in formaldehyde disinfection than is the moisture given off by the other methods.

As has already been stated, the temperature and humidity are of paramount importance in disinfecting with formaldehyde. On the other hand, its employment has the objections that have been mentioned in case of the retort and autoclave, namely, that special apparatus is required and time is consumed in evolving the formaldehyde. An important feature of this lamp generator is that there is no polymerization of formaldehyde into paraform following its use.

VII. FORMALIN ALUMINUM SULPHATE LIME METHOD.

This method of liberating formaldehyde gas from formalin was first described by Walker,[a] of the department of health, Brooklyn, N. Y.

---

[a] Walker, Henry G.: On a new method of generating formaldehyde gas for fumigating purposes. Journ. Am. Chem. Soc., vol. 24, No. 3, March, 1905.

He dissolves 20 to 25 pounds of commercial aluminum sulphate in 5 gallons of hot water and mixes this solution with 15 gallons of 40 per cent formaldehyde solution. Eight fluid ounces of this mixture and 1 pound of lime are used for 1,000 cubic feet. He recommends that the lime be used in small particles and that it should slake rapidly in cold water. We used the method practically as recommended by Walker.

Aluminum suphate was added to hot water in the proportion of 50 grams of the former to 100 c. c. of the latter. For an experiment 300 c. c. of this solution, 600 c. c. of formalin, and 2,000 grams of unslaked lime were usually used. Before using the lime it was broken into small particles and placed in a large bucket in the room. When ready to start, the formalin and aluminum sulphate solution were mixed and poured over the lime. The lime began to slake in a few minutes and continued about 20 minutes, during which time considerable formaldehyde, steam, etc., were given off. At the end of this time the process of liberation appeared to be completed.

Chemical determinations were made in the usual way, allowing about 40 minutes to elapse after starting an experiment before beginning to draw air from the room. As it was soon found that the percentage of formaldehyde liberated by this method is relatively low, only a few experiments were done. About 14 per cent was found, which is less than half that liberated by several of the other methods. After the experiment was over (2 hours) the lime was found completely slaked and in a perfectly dry state.

A summary of conditions and results is given in each of the following experiments performed for the purpose of determining the disinfecting value of this method:

EXPERIMENT NO. 38.

Formalin-aluminum sulphate-lime method.

Formalin, 600 c. c.
Solution of aluminum sulphate in water (proportion of 50 grams to 100 c. c. of water), 300 c. c.,
Unslaked lime (in small particles), 2,000 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Quantity of formaldehyde found per cubic foot, 0.01498 gram.
(Determination made by drawing air from room beginning 40 minutes after mixing the formalin-aluminum solution and lime.)
Yield of formaldehyde, 14 per cent.
Weather: Practically no wind; warm and rainy.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before mixing formalin, aluminum sulphate, and lime	87	66	8.92
After 10 minutes	87.5	85	11.67
After 20 minutes	87.5	83	11.39
After 2 hours	88.0	81	11.29

Time of exposure of organism counted from time of mixing the formalin-aluminum solution and lime.

EXPERIMENT NO. 38—Continued.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.					
		5	10	20	30	45	60
B. coli communis	Glass	+	–	–	–	–	–
B. subtilis	do				–	–	–
B. pyocyaneus	Filter paper	+	+	–	–	–	–
B. typhosus	do	+	+	–	–	–	–
B. coli communis	do	+	+	–	–	–	–
B. subtilis	do	+	+	+	+	–	–
B. coli communis	Wilson method	+	+	–	–	–	–

EXPERIMENT NO. 39.

Formalin-aluminum sulphate-lime method.

Formalin, 600 c. c.
Solution of aluminum sulphate in water (proportion of 50 grams to 100 c. c. of water), 300 c. c.
Water, 300 c. c.
Unslaked lime, 2,500 grams.
Capacity of room, 2,000 cubic foot.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Percentage yield of formaldehyde undetermined.
Weather: Light westerly wind, cloudy.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° *F.*	*Per cent.*	
Before mixing formalin, aluminum sulphate, and lime	71	58	4.78
After 10 minutes	72	82	6.98
After 20 minutes	72	86	7.31
After 3 hours	74	65	5.89

Time of exposure of organism counted from time of mixing the formalin-aluminum solution and lime.

[+ means growth; – no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.							
		10	20	30	45	60	90	120	180
B. pyocyaneus	Glass	+	+	–	–	–	–	–	–
B. coli communis	do	+	–	–	–	–	–	–	–
B. subtilis	do	+	+	+	+	+	+	–	–
B. typhosus	Filter paper	+	–	–	–	–	–	–	–
B. coli communis	do	+	+	–	–	–	–	–	–
B. subtilis	do	+	+	+	+	+	+	+	+

EXPERIMENT NO. 40.

Formalin-aluminum sulphate-lime method.

Formalin, 600 c. c.
Solution of aluminum sulphate in water (proportion of 50 grams to 100 c. c. of water), 300 c. c.
Unslaked lime (in small particles), 2,000 grams.
Capacity of room, 2,000 cubic feet.
Quantity of formaldehyde used per cubic foot, 0.107 gram.
Yield of formaldehyde undetermined.
Weather: Clear; light wind.

	Temperature.	Relative humidity.	Absolute humidity (grams per cubic foot).
	° F.	*Per cent.*	
Before mixing formalin, aluminum sulphate, and lime	69	47	3.63
After 10 minutes	69	67	5.17
After 3 hours	70	64	5.10

Time of exposure of organism counted from time of mixing the formalin-aluminum solution and lime.

[+ means growth; –, no growth.]

Organism.	How exposed.	Time of exposure in minutes, and results.							
		10	20	30	45	60	90	120	180
B. pyocyaneus	Glass	+	+	+	+	–	–	....	....
B. coli communis	do	+	+	+	+	+	+	+	....
B. subtilis	do	+	+	+	+	+	+	+	+
B. pyocyaneus	Wilson method	+	+	+	+	+	+	....	....
B. coli communis	Filter paper	+	+	+	+	+	+	+	....
B. subtilis	do	+	+	+	+	+	+	+	+

The percentage of formaldehyde found in experiment No. 38 was low; but, as the temperature and humidity were comparatively high, the organisms, including the spores of *B. subtilis*, were destroyed in rather a short time.

In experiment No. 39 the temperature and humidity were not so high as in the previous experiment, and consequently the killing power of the formaldehyde was much diminished. The spores of *B. subtilis* exposed on filter paper were not killed within 3 hours. There is certainly nothing in the results of this experiment to indicate that the addition of 300 c. c. of water was of any advantage for disinfection purposes.

A low humidity affects the germicidal power of the formaldehyde in this method to about the same extent as it does in the other methods. This is shown in experiment No. 40, humidity 47 per cent, in which the only organism killed was *B. pyocyaneus* exposed on glass. This method has nothing to recommend it especially for disinfection purposes unless it is the amount of moisture given off. This is about the same as with the formalin-permanganate method; but, as has been mentioned above, it is an open question what rôle this form of moisture plays in disinfecting with formaldehyde. The percentage of formaldehyde liberated by this method is less than half that obtained by the formalin-permanganate method. The latter method is also simpler and more easily conducted than the former. For practical disinfection some of the other methods, notably the formalin-permanganate, are superior to the formalin-aluminum-sulphate-lime method; consequently, further experiments with the latter method seemed unnecessary, although under favorable conditions it may be used with very good results.

EXPERIMENTS IN A LARGE BOTTLE WITH THE FORMALIN-PERMANGANATE METHOD.

As the changes in climatic conditions caused variations in the results of the experiments performed in the room, some experiments were performed in a large bottle, in which certain influences would be either eliminated or more amenable to control.

Furthermore, the special purpose of these experiments precluded the possibility of conducting them in a room of any character. This purpose may be briefly summarized as follows: (1) To determine with the formalin-permanganate method under different conditions the quantity of formaldehyde liberated and also the quantity left in the residue in the generator; (2) to determine the minimum relative humidity and lowest temperature at which formaldehyde disinfection is practicable.

Chemical determinations.—The chemical experiments were carried out in two bottles, one holding a trifle over 24 liters (0.85 cubit foot), the other very nearly 18 liters (0.63 cubic foot). The mouths of the bottles were 2.75 inches wide and were closed with tight-fitting corks, the pores of which were sealed with melted paraffin.

A porcelain crucible holding 24 c. c. was fitted into an excavated flat cork, which was suspended in a stirrup of thin twine.

The formalin was practically 40 per cent by volume, as determined by the Blank and Finkenheimer hydrogen dioxide method. The quantity to be used in an experiment, usually 0.7 c. c., was carefully measured from a slender pipette in which 0.1 c. c. made a volume 1.7 cm. long. The formalin was used undiluted in some experiments and in others a definite quantity of water was added to it before mixing with the permanganate.

I. EXPERIMENTS WITH UNDILUTED FORMALIN AND PERMANGANATE.

Method of procedure for determining the percentage of gas liberated.—About 40 c. c. of approximately decinormal potassium cyanide solution were measured from a burette into the bottle and about 50 c. c. of water added. The liquid was then rolled over the side of the bottle to produce a greater absorbing surface. Then the crucible containing the formaldehyde was suspended in the mouth of the bottle, the permanganate dropped in from a piece of glazed paper, the whole let down quickly into the bottle by a string, and the cork securely placed.

After a few seconds a vigorous action began, which seemed to be over in about 5 minutes. About 15 minutes, however, were allowed to elapse before the crucible was removed. By repeatedly rolling the cyanide solution over the side of the bottle the formaldehyde was soon absorbed (in about half an hour). After the odor of formaldehyde could not be detected the contents of the bottle were transferred by thorough washing to a 500 c. c. flask containing an excess of decinormal silver nitrate (about 10 c. c.) acidified with nitric acid. The volume was made up to the mark, the flask thoroughly shaken, 250 c. c. of the filtrate titrated with sulphocyanate solution, and calculation made as already described.

Method of procedure for the residue in the crucible.—After the crucible was removed, the contents were thoroughly extracted with water and filtered into a 250 c. c. flask. The volume was made up to the mark and 50 c. c. of this liquid were added to about 15 c. c. of the cyanide solution. After stirring, it was poured into an excess of acidified silver nitrate solution, which was stirred until the precipitate collected into a clot. The clear filtrate from the precipitate was then titrated with sulphocyanate solution for excess of silver.

TABLE 6.—*Results of experiments in glass bottles.*

	Experiment, date, etc.	Size of bottle, liters.	Formalin (40 per cent) by volume.	$KMnO_4$.	Formaldehyde found in bottle.	Formaldehyde found in residue.	Total of formaldehyde found in bottle and residue.	Remarks.
			c. c.	Gm.	Per ct.	Per ct.	Per ct.	
I	Aug. 15, 1905	24	1	0.5	33.03	....	....	$KMnO_4$ in small needle crystals.
II	Aug. 16, 1905	24	1	0.5	33.52	38.25	71.77	Do.
III	Aug. 18, 1905	18	1	0.5	32.47	40.35	72.82	Do.
IV	Aug. 17, 1905	18	0.7	0.35	35.48	33.86	69.34	$KMnO_4$ ground to moderately fine powder.
V	Aug. 18, 1905	18	0.7	0.35	36.53	35.20	71.73	Do.
VI	....do	24	1	0.5	....	35.11	....	Do.
VII	Aug. 21, 1905	18	0.7	0.35	37	32.20	69.20	Do.
VIII	Aug. 22, 1905	18	0.7	0.35	37.83	32.76	70.59	Do.
IX	Aug. 19, 1905	18	0.5	0.25	33.28	38.10	71.38	Do.
X	Aug. 25, 1905	18	0.7	0.4	34.37	25.16	59.53	Do.
XI	Aug. 28, 1905	18	1	0.39	25.20	42.79	67.99	Do.
XII	Jan. 3, 1906, 41 ° F.	18	0.7	0.35	30.84	39.09	69.93	Do.
XIII	Jan. 5, 1906, 41 ° F.	18	0.7	0.35	30	34.1	64.1	Do.
XIV	Feb. 8, 1906, 34 ° F.	18	0.7	0.35	30.1	34.15	64.16	Do.
XV	Feb. 3, 1906, 20 ° F.	18	0.7	0.35	28.26	....	....	Do.

Experiments I to XI, inclusive, Table 6, were done during warm weather (summer), and in all but X and XI the proportions used were 1 part by volume of formalin to 0.5 part by weight of permanganate, the same as was used in the experiments in Table 1. With this proportion the total amount of formaldehyde found in the air of the bottle and in the residue was fairly constant, considering the conditions of the experiments.

Evidently there is an advantage in using permanganate in powdered form, as is shown by comparing the results of IV, V, VI, VII, and VIII, with those of I, II, and III. No doubt the reason for this is that action takes place more quickly with production of a higher temperature, so that more formalin evaporates from the crucible and less remains with the residue.

The results in experiments I, II, and III, with an average of 33 per cent, are lower than the results in Table 1, which refer to experiments in charging the room of 2,000 cubic feet. This would be expected considering the small quantities used, whereby the quantity of heat produced is not so large and the cooling of the generator is more rapid. In the experiments where powdered permanganate was used, the percentage yield is higher and in line with the results in Table 1.

In experiment IX, where only 0.5 c. c. of formalin was used, the result is low; but this is probably due to the fact that the quantity of formalin is too small to produce as vigorous an action as did the other cases.

In experiment X the proportion of permanganate was increased, with the result that the amount of the formaldehyde sent out into the bottle was not affected much, but considerably less remained with the residue.

In experiment XI the permanganate was decreased to the proportion used by Evans (Maine State board of health) in the report on this method. The result shows a considerable decrease in the formaldehyde sent out into the bottle and large increase in the amount remaining in the residue.

Experiments XII to XV inclusive were performed during cold weather (winter), the results of which demonstrate that the percentage of formaldehyde actually given off during cold weather does not differ very materially from that given off during warm weather.

In these experiments any paraformaldehyde, whether given off as such or resulting from polymerization of the formaldehyde after it was given off, is included in the percentage of formaldehyde liberated, since paraformaldehyde enters into union with the cyanide in the same manner as does formaldehyde gas.

The results of these experiments also show that the reduced yield of formaldehyde obtained in the room when working at a cold temperature was not due so much to the cold causing a reduction in the quantity of formaldehyde given off as to polymerization of the liberated formaldehyde resulting from the cold.

II. EXPERIMENT WITH DILUTED FORMALIN AND PERMANGANATE.

Two experiments were made with 0.6 c. c. formalin diluted with 0.3 c. c. water and 0.375 gm. of powdered permanganate; these are the same proportions as used in most of the experiments in Table 2. The procedure was the same as described above.

Experiment.	Formaldehyde found in bottle.	Formaldehyde found in residue.	Total found.
	Per cent.	*Per cent.*	*Per cent.*
I	31.00	34.23	65.23
II	32.16	34.04	66.20

The percentage yield was about the same as that given in Table 2, but less than that in the previous experiments (Table 6) with undiluted formalin. The formaldehyde remaining with the residue was about the same as in the previous experiments where powdered permanganate was used. The total formaldehyde found was less than in the experiments with undiluted formalin, thus indicating a greater destruction of formaldehyde.

III. EXPERIMENTS ON DRYING FORMALDEHYDE GAS BY ANHYDROUS CALCIUM CHLORIDE.

The bottle was charged as in the previous cases, using 0.7 c. c. of formalin and 0.35 gm. of powdered permanganate. The crucible was removed between 15 and 20 minutes after it had been introduced into the bottle.

The drying apparatus consisted of a curved tray, made of a piece of sheet zinc about 1.75 inches wide, 10 inches long, and 1.25 inches deep. One end of the tray was closed by bringing the edges together; the other end was curved around a wooden handle, which fitted into a hole in a cork. The latter fitted the mouth of the bottle.

After removing the crucible the bottle was laid on its side. Granulated calcium chloride was placed in the tray in a layer about an inch deep; then as quickly as possible the stopper of the bottle was removed and the tray introduced and held in place by means of the stopper attached to its handle, which fitted the neck of the bottle.

After 1 hour, which seemed ample time to thoroughly dry the atmosphere in the bottle because of the large surface of calcium chloride exposed, the stopper and tray were removed and replaced by the first one.

Before introducing the calcium chloride the inner surface of the bottle was covered with moisture. This disappeared in about 15 or 20 minutes after the drying apparatus was introduced.

Naturally a little formaldehyde was lost by displacement and diffusion when the drying tray was introduced and withdrawn. This loss could not have been more than a small fraction of the amount of formaldehyde in the bottle when the crucible was removed.

After withdrawing the drying apparatus, potassium cyanide solution was measured into the bottle from a burette through an inch hole in the stopper. To prevent loss, the burette tip was passed through a small hole in a flat cork, which in turn fitted into the hole of the stopper, thus closing the mouth of the bottle. After removing the burette, the hole in the stopper was properly closed with a cork. The subsequent procedure was exactly like that described above (p. 66).

The formaldehyde left in the residue in the crucible was also determined in the manner already described (p. 67).

TABLE 7.—*Experiments on drying formaldehyde gas in 18-liter bottle by calcium chloride.*

Experiment.	Formalin 40 per cent by volume.	$KMnO_4$ powdered.	Time of drying.	Formaldehye found in bottle.	Formaldehyde found in residue.
	c. c.	*gm.*		*Per cent.*	*Per cent.*
I	0.7	0.35	1 hour	21.12	33.86
II	.7	.35	1 hour	20.06	32.52
III	.7	.35	2 hours	14.76	35.87

Since the bottle was charged in precisely the same manner and with the same quantities as in experiments IV, V, VII, and VIII, Table 6, we may assume that the average of these four results, namely, 36.71 per cent is the quantity of formaldehyde in the bottle in the present experiments before drying. The results of experiments I and II, Table 7, are fairly close, the average being 20.59 per cent. The difference, 16.12 per cent is the loss, due mainly to drying and, in a small degree, to escape by introduction and removal of the drying apparatus. It is probable that more than one-third of the formaldehyde disappeared by the drying during 1 hour. By 2 hours' drying still more disappeared, as shown in experiment III.

The question as to what becomes of the formaldehyde that disappears is an interesting one; but it was not investigated. After the calcium chloride had been removed from the bottle it was stored in a closed jar. This jar had a strong odor of the gas. It is probable that some of the formaldehyde was held as such in the pores of the calcium chloride granules. Whether the latter also had a polymerizing effect on the formaldehyde we are not able to say.

The quantities of formaldehyde found remaining with the residue in the porcelain crucible (average 34.08 per cent) agree fairly well with the results in experiments IV, V, VII, and VIII, Table 6 (average 33.50 per cent).

IV. HUMIDITY AND TEMPERATURE IN FORMALDEHYDE DISINFECTION.

The influence and importance of humidity and temperature became very evident in many of the experiments, but owing to uncontrollable circumstances in the room their minimum limitations, particularly of the humidity, could not be closely approximated. It will readily be seen that it is of more practical importance to know this in case of the humidity than of the temperature, since the former is subject to wide variations even within a few hours. For instance, the humidity of the atmosphere may be 90 per cent at 8 a. m., 30 per cent at 2 p. m., and 90 per cent again at 8 p. m. of the same day. Such variations in temperature seldom, if ever, occur in so short a time. As we had observed in the room experiments that formaldehyde is inefficient as a disinfectant when employed during cold weather, most of the experiments for determining the minimum limitations of humidity were done at ordinary summer temperatures. For conducting the experiments a large bottle holding 24 liters (0.85 cubic foot) was used. As this bottle, the mode of setting off the formalin and permanganate, the process of drying with calcium chloride, etc., have already been fully described (p. 66), only the more important details will be given here.

The quantities of formalin and permanganate used were in the proportion of 1 c. c. of the former to 0.5 gram of the latter. The formalin contained about 40 per cent of formaldehyde by volume. The result of mixing the formalin and permanganate in the above proportions was a vigorous reaction, which, in addition to evolving formaldehyde, gave off rather a large quantity of aqueous vapor, which settled on the sides of the bottle to such an extent as to make the glass almost nontransparent.

A few experiments easily demonstrated that with a high temperature, say 80° F., even a small percentage of formaldehyde with a large quantity of moisture, is a most powerful surface disinfectant, killing 40-day old spores of *Bacillus subtilis* in a few minutes.

The object then was to remove the moisture in the bottle by introducing a metal tray containing anhydrous calcium chloride, after which a definite quantity of water could be injected in the form of aqueous vapor.

Soon after introducing the calcium chloride the moisture from the sides of the bottle would disappear. The atmosphere in the bottle was assumed to be in a dry condition after about 1 hour, and the tray containing the calcium chloride was then removed. A thermometer was then introduced and the temperature in the bottle determined. Having ascertained this, the quantity of water necesssary to give a certain percentage of relative humidity was easily estimated by referring to the pscychrometric tables for obtaining the vapor pressure, relative humidity, and temperature of the dew-point, prepared by the weather Bureau, United States Department of Agriculture.

The desired quantity of water was measured in a graduated pipette and placed in a test tube, the open end of which had been drawn out into a small tube about 0.5 centimeter in diameter; to be more accurate, the tube with its contents was then carefully weighed. The small end of the test tube was introduced into the bottle and the other end gently heated. This caused the water to pass into the bottle in the form of steam. After vaporizing the water, the tube was left in place until it had cooled; this caused some of the moisture to recondense on the sides of the tube. The tube was removed and again carefully weighed, the difference in the two weighings giving the quantity of water actually injected in the form of aqueous vapor.

This factor, and the temperature, being known, the percentage of relative humidity was easily computed from the tables referred to above (p. 72). The exact degree of relative humidity desired was often not easily obtained, as it was difficult to inject the precise quantity of water wanted, and, furthermore, the temperature was subject to change. The slightest variation in either 1 of these 2 factors gives a different percentage of relative humidity.

For making the bacteriological exposures, 24-hour old agar cultures of *B. coli communis* and spores of *B. subtilis*, grown about 40 days on agar slants, were used. An emulsion of the organisms in distilled water was made, small slips of filter paper about 0.5 cm. square were moistened with the emulsion and placed in the incubating room for about 1 hour in order to thoroughly dry them.

The slips of filter paper were then placed in a small copper-wire tray, previously sterilized, and exposed to the action of the formaldehyde in the bottle by introducing through a hole in the large cork stopper. This tray was provided with a tight-fitting cork on the near end, so that when the tray was in place in the bottle the cork would close the hole through which it was introduced. This arrangement made it possible to withdraw the tray wholly or partly when desired. The slips of filter paper were taken out at definite intervals and planted in tubes of nutrient bouillon. The tubes were then placed in the incubating room at a temperature of 37° C. and the results recorded after 10 days' incubation.

TABLE 8

Experiments in a 24-liter bottle.
Formalin-permanganate method.
Formalin 1 c. c. and permanganate 0.5 gram.
Moisture removed by calcium chloride.
Varying quantities of moisture introduced.
Ordinary summer temperatures.
B. coli communis exposed on filter paper.

[+ means growth; — no growth.]

Temperature at time of experiment.	Quantity of water injected as aqueous vapor.		Time of exposure in minutes, and results.									
	Absolute humidity, grains per cubic foot.	Relative humidity.	5	10	15	20	30	45	60	75	90	120
75.0	7.43	80	+	—	—	—	—	—	—	—	—	—
85.0	8.1	64	....	—	—	—	—	—	—	—	—	—
83.0	7.21	60	+	+	+	+	—	—	—	—	—	—
79.0	6.28	59	+	+	+	+	—	—	—	—	—	—
69.0	4.32	56	+	+	+	+	—	—	—	—	—	—
73.0	4.73	54	+	+	+	+	—	—	—	—	—	—
88.0	7.54	54	+	+	+	+	+	—	—	—	—	—
86.0	6.71	51	+	+	+	+	+	—	—	—	—	—
80.0	5.43	50	+	+	+	+	+	+	—	—	—	—
66.5	3.5	49	+	+	+	+	+	+	+	+	+	+
86.0	6.37	49	+	+	+	+	+	+	+	+	....	....
78.0	4.9	48	+	+	+	+	+	+	+	....	....	....
80.0	5.0	46	+	+	+	+	+	+	+	+	+	+
84.0	3.64	30	+	+	+	+	+	+	+	+	+	+

TABLE 9.

Experiments in a 24-liter bottle.
Formalin-permanganate method.
Formalin 1 c. c. and permanganate 0.5 gram.
Moisture removed by calcium chloride.
Varying quantities of moisture introduced.
Ordinary summer temperatures.
B. subtilis exposed on filter paper.

[+ means growth; — no growth.]

Temperature at time of experiment.	Quantity of water injected as aqueous vapor.		Time of exposure in minutes, and results.									
	Absolute humidity, grains per cubic foot.	Relative humidity.	5	10	15	20	30	45	60	75	90	120
75.0	7.43	80	+	+	+	—	—	—	—	—	—	—
83.0	7.21	60	+	+	+	+	—	—	—	—	—	—
79.0	6.28	59	+	+	+	+	+	+	—	—	—	—
69.0	4.32	56	+	+	+	+	+	+	—	—	—	—
73.0	4.73	54	+	+	+	+	+	—	—	—	—	—
88.0	7.54	54	+	+	+	+	+	+	+	....	....	....
86.0	6.82	52	+	+	+	+	+	+	+	....	....	....
77.0	5.13	52	+	+	+	+	+	+	+	+	+	+
80.0	5.43	50	+	+	+	+	+	+	+	....	....	....
86.0	6.37	49	+	+	+	+	+	+	+	+	+	+
66.5	3.5	49	+	+	+	+	+	+	+	+	+	+
78.0	4.9	48	+	+	+	+	+	+	+	+	+	+
80.0	5.0	46	+	+	+	+	+	+	+	+	+	+
84.0	3.64	30	+	+	+	+	+	+	+	+	+	+

TABLE 10.

Experiments in a 24-liter bottle.
Formalin-permanganate method.
Formalin 0.5 c. c. and permanganate 0.25 gram.
Moisture removed by calcium chloride.
Varying quantities of moisture introduced.
Ordinary summer temperatures.
B. coli communis exposed on filter paper.

[+ means growth; — no growth.]

Temperature at time of experiment.	Quantity of water injected as aqueous vapor.		Time of exposure in minutes, and results.									
	Absolute humidity, grains per cubic foot.	Relative humidity.	5	10	15	20	30	45	60	75	90	120
77.0	6.17	62	+	+	—	—	—	—	—	—	—	—
77.5	5.44	54	+	+	+	+	—	—	—	—	—	—
75.0	4.64	50	+	+	+	+	+	+	+	+	+	+

TABLE 11.

Experiments in a 24-liter bottle.
Formalin-permanganate method.
Formalin 0.5 c. c. and permanganate 0.25 gram.
Moisture removed by calcium chloride.
Varying quantities of moisture introduced.
Ordinary summer temperatures.
B. subtilis exposed on filter paper.

[+ means growth; — no growth.]

Temperature at time of experiment.	Quantity of water injected as aqueous vapor.		Time of exposure in minutes, and results.									
	Absolute humidity, grains per cubic foot.	Relative humidity.	5	10	15	20	30	45	60	75	90	120
77.0	6.17	62	+	+	+	+	+	—	—	—	—	—
77.5	5.44	54	+	+	+	+	+	+	—	—	—	—
75.0	4.64	50	+	+	+	+	+	+	+	+	+	+

It will be observed from the above experiments that with a relative humidity between 50 and 60 per cent both *B. coli communis* and *B. subtilis* were killed within a reasonably short time, but below 50 per cent neither organism was killed within 2 hours.

These results demonstrate the important part that humidity plays in formaldehyde disinfection and that the minimum limit lies between 50 and 60 per cent. This is a slightly smaller percentage than was found necessary in the room experiments to accomplish disinfection; but of course the conditions in the bottle were unusually favorable for germicidal action and slightly better results would therefore be expected.

The different temperatures at which the experiments were performed may account for some of the little variations in the results of the above tables (8–11).

The question might arise whether absolutely all of the moisture was removed from the bottle by the calcium chloride after 1 hour exposure; but from the known chemical affinity of this substance for water it would seem that all of it was absorbed.

If all the moisture was removed from the bottle by the calcium chloride, the idea that once prevailed, namely, that formaldehyde can not exist in a perfectly dry state, is disproved.

A comparison of the percentage of humidity required in these experiments with that required in the room experiments (p. 29), would seem to indicate that the moisture given off by the process of liberation in the latter experiments is of much less importance than the moisture in the air before the experiment is started.

It is not so much the absolute as the relative humidity that is necessary in formaldehyde disinfection. For example, the action of an ordinary quantity of formaldehyde is strongly germicidal in the presence of 5.586 grains (0.36 gram) of moisture per cubic foot at a temperature of 70° F. (relative humidity 70 per cent), but is almost inert in the presence of 6.85 grains (0.443 gram) per cubic foot at a temperature of 95° F. (relative humidity 40 per cent).

By referring to Table 7 it will be seen that a large quantity of formaldehyde was removed from the bottle in the process of drying. It may be assumed from our experimental determinations that before drying, the bottle contained 36.71 per cent of the formaldehyde theoretically present in the quantity of formalin used. By drying 1 hour with calcium chloride the quantity of formaldehyde was reduced to about 20.5 per cent and after 2 hours to 14.76 per cent.

To show the germicidal power of a reduced quantity of formaldehyde in the presence of a high humidity and temperature two experiments were done, using one-fourth the original quantities of formalin and permanganate used in Tables 8 and 9, namely, formalin 0.25 c. c. and permanganate 0.125 gram.

In one experiment with a relative humidity of 61 per cent, temperature 26° F., *B. coli communis* was killed within 30 minutes. In the other, with relative humidity of 59 per cent, temperature 60° F., the same species was killed within the same time. *B. subtilis*, exposed in both experiments at the same time as *B. coli communis*, were not killed within 2 hours.

Two more experiments were performed in which one-eighth the original quantities of formalin and permanganate were used, namely, formalin 0.125 c. c. and permanganate 0.0625 gram. In one, with a relative humidity of 75 per cent, temperature 74° F., *B. coli communis* was killed within 30 minutes, while in the other, with a relative humidity of 59 per cent, temperature 70° F., the same species was killed within one hour. *B. subtilis*, exposed at the same time as *B. coli communis*, was not killed in either experiment within 2 hours.

These results indicate that a reduction in percentage of formaldehyde diminishes its killing power proportionately more for spore bearing than nonspore-bearing organisms.

An experiment was performed in which the moisture was absorbed by 1 hour exposure of calcium chloride, after which no moisture was introduced. *B. coli communis* and *B. subtilis* exposed for 24 hours under these conditions grew as well when planted in boullion as did the controls. The temperature at time of exposing the organisms in this experiment was 83° F.

In another experiment, in which the bottle was charged with formalin 1 c. c. and permanganate 0.5 gram and dried 1 hour with calcium chloride, slips of filter paper were moistened with an emulsion of *B. coli communis* and *B. subtilis*, respectively, and exposed while the slips were still in a very moist condition. *B. coli communis* was killed within 10 minutes and *B. subtilis* within 30 minutes. This simply shows that in the presence of the moisture on the slips the formaldehyde exerted a germicidal action.

In order to demonstrate the influence of cold upon the germicidal properties of formaldehyde, a number of experiments were conducted at or below a temperature of 60° F. Different percentages of humidity were also used.

The method of setting off the formalin and permanganate, the drying by calcium chloride, and the introduction of the moisture have been described (p. 66).

A summary of conditions and results are given in the following table:

TABLE 12.

Experiments in a 24-liter bottle; formalin-permanganate method.

Formalin 1 c. c., permanganate 0.5 gram.
Moisture removed by calcium chloride.
Varying quantities of moisture introduced.
Temperature at or below 60° F.
B. coli communis and spores of *B. subtilis* exposed on filter paper.

[+ means growth, − no growth.]

Results with *B. coli communis*, and time of exposure in minutes.										Temperature.	Relative humidity.	Results with spores of *B. subtilis*, and time of exposure in minutes.									
5	10	15	20	30	45	60	75	90	120			5	10	15	20	30	45	60	75	90	120
										° *F.*	*Per c*										
+	+	−	−	−	−	−	−	−	−	60	8	+	+	+	+	+	+	+	−	−	−
+	+	−	−	−	−	−	−	−	−	60	8	+	+	+	+	+	+	+	+	−	−
+	+	+	−	−	−	−	−	−	−	60	8	+	+	+	+	+	+	+	+	−	−
+	+	−	−	−	−	−	−	−	−	60	6	+	+	+	+	+	+	+	....	....	....
+	+	+	+	−	−	−	−	−	−	60	6	+	+	+	+	+	+	+	+	+	....
+	+	+	+	−	−	−	−	−	−	60	5	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	−	−	−	−	−	60	5	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	−	−	−	−	60	5	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	−	−	−	−	53	[illegible]	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	−	−	−	−	50	[illegible]	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	−	−	−	−	50	[illegible]	+	+	+	+	+	+	+	+	+	−
+	+	+	+	+	+	+	−	−	−	46	[illegible]	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	−	−	40	[illegible]	+	+	+	+	+	+	+	+	+	+

Comparison of Tables 8, 9, and 12 shows that the time required for germicidal action is materially increased by a reduction in temperature, even though it is not below 60° F.

This is more noticeable with *B. subtilis* than with *B. coli communis*. At a lower temperature the killing power of the formaldehyde becomes so limited that only a few experiments were performed at a temperature lower than 60° F. With a temperature of 40° F. and humidity of 95 per cent, *B. coli communis* was killed within an hour and a half; but, considering the method of exposing, the organism, and the fact that as high humidity as that is rarely observed at that temperature except under artificial conditions the results can not be considered satisfactory.

DISINFECTION OF SLEEPING CARS WITH FORMALDEHYDE GAS.

These experiments were conducted for the purpose of determining the practical value and efficiency of the formalin-permanganate method of liberating formaldehyde gas in the disinfection of sleeping cars.

For comparison, experiments were also done with other well-known methods of evolving the gas for disinfection purposes. The work was done in the cars of the Pullman Company at their local yards. We are indebted to the officials of this company for the use of the cars and their cooperation and assistance in carrying out this work. The cars were selected at random, regardless of when they had been occupied; usually they had been used the previous night. Often the cars were shifted about in the yard during the experiment. Although the size of the different cars varied slightly, for our purposes their air space was approximately 4,500 cubic feet.

Usually 2,000 c. c. of formalin, containing 35.66 per cent by volume of formaldehyde, were used for an experiment. Before starting all berths were let down, inside doors opened, and windows and ventilators closed. When practicable, the lamp ventilators were closed on the inside with cotton waste; otherwise they were closed on top of the car by wrapping cloths around them. No pasting of cracks was done.

All organisms used, except *B. tuberculosis*, were grown, prepared, and exposed by the Wilson method, as described on page 17. For *B. tuberculosis*, tuberculous sputum, showing on examination many tubercule bacilli, was used. Exposures of this were made by the Wilson method and on small pieces of carpet, using in each case a drop or two of the sputum. For convenience and safety, the pieces of carpet were carried in a Petri dish and exposed in the car by simply removing the top of the dish. After preparing the exposures they were dried in the incubator at a temperature of 37° C. for 1 hour, and, as the laboratory is some distance from the car yards, about another hour elapsed before the exposures were made. They were variously placed in the car for exposure, the exact location being recorded in each case.

Different lengths of exposure might have been tried, but in our experiments 2 hours was chosen. Therefore, the car in all experiments was opened after 2 hours from the time of beginning to liberate the gas. The organisms were removed from the car as soon thereafter as possible, usually about 15 minutes, and taken to the laboratory. With the exception of *B. tuberculosis*, they were planted in nutrient bouillon, placed in the incubating room, and the results recorded, as has been described (p. 18).

In case of *B. tuberculosis*, the pieces of carpet and slips of filter paper (Wilson method) were washed in bouillon and the washings injected intraperitoneally into guinea pigs. Control pigs were also inoculated at the same time. The pigs were weighed before and at definite intervals after inoculation; those that did not die within about 3 months were chloroformed. A careful post-mortem examination was made of all. The gain or loss of the chloroformed pigs is given in each experiment; the figures refer to the weight of the pig when inoculated as compared with its weight when chloroformed.

Although the cars seemed comparatively tight, the small percentage of formaldehyde gas found in the cars on opening showed that much of it had escaped during the 2 hours. The temperature and relative humidity were taken just before starting an experiment and again as soon after it was over as possible. The second reading, however, indicates more the condition of the outside atmosphere than any change resulting from the experiment, as the car was then being aired.

I. FORMALIN-PERMANGANATE METHOD.

Formalin and permanganate were used in these experiments in the same proportions as given on page 20, namely, 1 c. c. of the former to 0.5 gram of the latter. The ordinary commercial crystals of permanganate were used without further powdering. The formalin and permanganate were mixed in galvanized-iron buckets, one being used for every 500 c. c. of formalin, and placed in the car as described in each experiment. As the quantities of the formalin and permanganate used, the condition of the weather, including temperature, relative humidity, winds, etc., are given in each individual experiment, further description here is unnecessary. The experiments are as follows:

EXPERIMENT No. 41.

Formalin 2,000 c. c., permanganate 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.
Weather: Clear; light southerly wind.
Temperature in car at beginning of experiment, 83° F.
Relative humidity in car at beginning of experiment, 78 per cent.
Time of exposure, 2 hours (11.15 a. m. to 1.15 p. m.).
Temperature in car after opening and airing 15 minutes, 87° F.
Relative humidity in car after opening and airing 15 minutes, 62 per cent.

[+ means growth; — no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	—
	Smoking room	—
	Drawing-room	—
	Floor main sleeping compartment	—
	Berth, lower No. 6	—
B. diphtheriæ	Smoking room	—
	Drawing-room	—
	Floor main sleeping compartment	—
	Berth, upper No. 4	—
	Berth, lower No. 8	—
B. typhosus	Toilet room	—
	Smoking room	—
	Drawing-room	—
	Floor main sleeping compartment	—
	Berth, upper No. 6	—
B. subtilis	Smoking room	—
	Drawing-room	—
	Berth, upper No. 10	—
	Berth, lower No. 8	—
B. tuberculosis	Smoking room	—
	Sputum exposed and injected into guinea pig; pig chloroformed 100th day; increased 255 grams in weight; no lesions of tuberculosis.	
	Floor of main sleeping compartment	—
	Sputum exposed and injected into guinea pig; pig chloroformed 100th day; increased 235 grams in weight; no lesion of tuberculosis.	
	Control pig died of tuberculosis 45th day.	

EXPERIMENT NO. 42.

Formalin 2,000 c. c., permanganate 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.

Weather: Clear; very light breeze; light rain previous night.

Temperature in car at beginning of experiment, 80° F.

Relative humidity in car at beginning of experiment, 81 per cent.

Time of exposure, 2 hours (11.20 a. m. to 1.20 p. m.).

Temperature in car after opening and airing 15 minutes, 83° F.

Relative humidity in car after opening and airing 15 minutes, 72 per cent.

[+ means growth; — no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	—
	Smoking room	—
	Drawing-room	+
	Floor main sleeping compartment	—
	Berth, upper No. 2	—
B. diphtheriæ	Smoking room	—
	Drawing-room	—
	Floor main sleeping compartment	—
	Berth, upper No. 10	—
	Berth, lower No. 4	—
B. yphosus	Toilet room	—
	Smoking room	—
	Drawing-room	—
	Floor main sleeping compartment	—
	Berth, lower No. 9	—
B. subtilis	Smoking room	—
	Toilet room	....
	Drawing-room	—
	Berth, upper No. 4	+
B. tuberculosis	Smoking room	—
	Sputum exposed and injected into guinea pig; pig chloroformed 103d day; increased 225 grams in weight; no lesions of tuberculosis.	
	Floor of main sleeping compartment	—
	Sputum exposed and injected into guinea pig; pig chloroformed 103d day; increased 260 grams in weight; no lesion of tuberculosis.	
	Control pig died of tuberculosis 45th day.	

EXPERIMENT NO. 43.

Formalin 2,000 c. c., permanganate 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.

Weather: Clear and dry, with practically no wind.

Temperature in car at beginning of experiment, 77.5° F.

Relative humidity in car at beginning of experiment, 69 per cent.

Time of exposure, 2 hours (11.45 a. m. to 1.45 p. m.).

Temperature in car after opening and airing 15 minutes, 83° F.

Relative humidity in car after opening and airing 15 minutes, 53 per cent.

[+ means growth; — no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	—
	Smoking room	—
	Drawing-room	—
	Berth, upper No. 9	—
	Berth, lower No. 8	—
B. diphtheriæ	Toilet room	—
	Smoking room	—
	Drawing-room	—
	Floor main sleeping compartment	—
	Berth, lower No. 6	—
B. pyocyaneus	Toilet room	—
	Smoking room	—
	Drawing-room	—
B. subtilis	Toilet room	—
	Smoking room	—
	Drawing-room	+
	Berth, lower No. 2	+

EXPERIMENT NO. 44.

Formalin, 2,000 c. c.; permanganate, 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alley way at each end.

Weather: Very cloudy and few drops of rain falling at times; no wind.

Temperature in car at beginning of experiment, 75° F.

Relative humidity in car at beginning of experiment, 82 per cent.

Time of exposure, 2 hours (12 m. to 2 p. m.) .

Temperature in car after opening and airing 20 minutes, 81° F.

Relative humidity in car after opening and airing 20 minutes, 72 per cent.

[+ means growth; — no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	—
	Smoking room	—
	Drawing-room	—
	Berth, upper No. 2	—
	Berth, lower No. 5	—
B. typhosus	Toilet room	—
	Drawing-room	—
	Berth, upper No. 6	—
	Berth, lower No. 1	—
	Berth, lower No. 12	—
B. pyocyaneus	Toilet room	—
	Smoking room	—
	Drawing-room	—
	Berth, upper No. 2	—
	Berth, lower No. 5	—
Staphylococcus pyogenes aureus.	Toilet room	—
	Smoking room	—
	Drawing-room	—
	Floor, main sleeping compartment	—
	Berth, upper No. 1	—
	Berth, lower No. 6	—
B. subtilis	Smoking room	+
	Drawing-room	+
	Berth, upper No. 2	+
	Berth, lower No. 12	+

EXPERIMENT NO. 45.

Formalin, 2,000 c. c.; permanganate, 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alley way at each end.
Weather: Clear and dry, with light variable wind.
Temperature in car at beginning of experiment, 74° F.
Relative humidity in car at beginning of experiment, 65 per cent.
Time of exposure, 2 hours (11.30 a. m. to 1. 30 p. m.).
Temperature in car after opening and airing 15 minutes, 82° F.
Relative humidity in car after opening and airing 15 minutes, 58 per cent.

[+ means growth; – no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	–
	Smoking room	–
	Drawing-room	–
	Floor, main sleeping compartment	+
	Berth, lower No. 10	–
B. diphtheriæ	Toilet room	–
	Smoking room	–
	Drawing-room	–
	Floor, main sleeping compartment	–
	Berth, upper No. 8	–
B. typhosus	Toilet room	–
	Smoking room	–
	Drawing-room	–
	Berth, upper No. 4	–
	Berth, upper No. 10	–
B. subtilis	Smoking room	–
	Toilet room	–
	Drawing-room	+
	Berth, upper No. 8	–
	Berth, upper No. 4	+
B. tuberculosis	Smoking room	–
	Sputum exposed and injected into guinea pig. Pig chloroformed 105th day; increased 325 grams in weight; no lesions of tuberculosis.	
	Floor of main sleeping compartment	–
	Sputum exposed and injected into guinea pig; pig chloroformed 105th day; increased 245 grams in weight; no lesion of tuberculosis.	
	Drawing-room	–
	Sputum exposed and injected into guinea pig; pig chloroformed 105th day; increased 270 grams in weight; no lesions of tuberculosis.	
	Control guinea pig died of tuberculosis 45th day.	

EXPERIMENT NO. 46.

Formalin, 2,000 c. c.; permanganate, 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment and one in the alleyway at each end.

Weather: Clear, practically no wind.

Temperature in car at beginning of experiment, 73° F.

Relative humidity in car at beginning of experiment, 61 per cent.

Time of exposure, 2 hours (11.45 a. m. to 1.45 p. m.).

Temperature in car after opening and airing 15 minutes, 83° F.

Relative humidity in car after opening and airing 15 minutes, 45 per cent.

[+ means growth; — no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	—
	Drawing-room	—
	Floor, main sleeping compartment	—
	Berth, upper No. 8	—
	Berth, lower No. 9	—
B. diphtheriæ	Toilet room	—
	Drawing-room	—
	Floor, main sleeping compartment	—
	Berth, lower No. 12	—
	Berth, upper No. 8	—
B. typhosus	Toilet room	—
	Drawing-room	—
	Floor, main sleeping compartment	—
	Berth, lower No. 6	—
	Berth, upper No. 10	—
B. subtilis	Toilet room	+
	Drawing-room	+
	Floor, main sleeping compartment	+
	Berth, upper No. 4	+
	Berth, lower No. 4	+
B. tuberculosis	Floor, main sleeping compartment Sputum exposed by Wilson method and injected into guinea pig; pig chloroformed 25th day; increased 250 grams in weight; lesions of tuberculosis in spleen and lymph glands.	+
	Floor, main sleeping compartment Sputum exposed on carpet and injected into guinea pig; pig chloroformed 95th day; lesions of tuberculosis in spleen and lungs. Control guinea pig died of tuberculosis 60th day.	+

Under ordinary conditions in disinfecting sleeping cars the formalin-permanganate method practically accomplishes complete surface disinfection when the weather is warm and the relative humidity high. The exact temperature and percentage of relative humidity necessary can not be stated within a few degrees, but it will be seen from the experiments that when the temperature is above 74° F. and the relative humidity above 61 per cent nearly all the nonspore-bearing organisms exposed to the action of the gas were killed within 2 hours.

Frequently old *subtilis* spores were killed in the short exposure of 2 hours, as will be seen in the experiments. Most of the tubercle bacilli exposed to the action of the gas were also killed in this time. In a few instances tubercle bacilli were not killed, but they were diminished in virulence. It is probable that a longer exposure than 2 hours would be more effective in this regard.

In the following experiments (Nos. 47 to 54, inclusive) it will be seen that when the temperature is relatively cold or the humidity comparatively low the formaldehyde gas fails to kill many of the organisms exposed.

It is especially interesting to note in the following experiments that while the results can not be considered satisfactory from a practical standpoint, because many of the organisms exposed escaped destruction, nevertheless the interesting point is brought out that organisms exposed in the toilet room of the sleeping car were almost invariably killed. No doubt the reason for this is the greater amount of moisture in the toilet room owing to the water present. As the toilet room is probably more apt to be infected than any other part of the coach, this is a matter of considerable practical value.

EXPERIMENT NO. 47.

Formalin 1,500 c. c., permanganate 750 grams, equally distributed in three iron buckets, one of which was placed in the main sleeping compartment of car and one in the alleyway at each end.
Weather: Clear, light variable wind.
Temperature in car at beginning of experiment, 78° F.
Relative humidity in car at beginning of experiment 63 per cent.
Time of exposure, 2 hours (12 m. to 2 p. m.).
Temperature in car after opening and airing 15 minutes, 83° F.
Relative humidity in car after opening and airing 15 minutes, 58 per cent.

[+ means growth; – no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	–
	Smoking room	–
	Drawing-room	–
	Berth, upper No. 3	–
	Berth, lower No. 6	+
B. typhosus	Toilet room	–
	Smoking room	–
	Drawing-room	–
	Floor main sleeping compartment	–
	Berth, upper No. 12	–
	Berth, lower No. 11	–
	Berth, lower No. 3	–
B. pyocyaneus	Toilet room	–
	Smoking room	–
	Drawing-room	–
	Floor main sleeping compartment	+
	Berth, upper No. 3	+
	Berth, upper No. 8	–
	Berth, lower No. 4	–
	Berth, lower No. 11	–
Staphyllococcus pyogenes aureus.	Toilet room	–
	Smoking room	–
	Berth, upper No. 6	+
	Berth, upper No. 12	+
	Berth, lower No. 3	–
B. subtilis	Smoking room	+
	Berth, upper No. 11	+
	Berth, lower No. 3	+

It is worthy of note here that although only half the quantities of formalin and permanganate were used in the preceding experiment as in the following one, the germicidal results in the former were better than in the latter. As the temperature was practically the same in the two experiments the difference in results is no doubt due to the higher humidity in the former experiment. This shows the importance of humidity in disinfecting with formaldehyde gas, and, further, that regardless of the quantity of gas present the humidity should not be below a certain percentage.

EXPERIMENT NO. 48.

Formalin 3,000 c. c., permanganate 1,500 grams, equally distributed in six iron buckets, four of which were placed in the main sleeping compartment of car and one in the alleyway at each end.
Weather: Clear and dry, light wind.
Temperature in car at beginning of experiment, 77.5° F.
Relative humidity in car at beginning of experiment, 58 per cent.
Time of exposure, 2 hours (11.20 a. m. to 1.20 p. m.).
Temperature in car after opening and airing 20 minutes, 83° F.
Relative humidity in car after opening and airing 20 minutes, 48 per cent.

[+ means growth; — no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	–
	Drawing-room	+
	Floor main sleeping compartment	–
	Berth, upper No. 10	+
	Berth, lower No. 6	–
B. diphtheriæ	Drawing-room	–
	Berth, upper No. 6	–
	Berth, lower No. 10	+
B. typhosus	Toilet room	–
	Drawing-room	+
	Berth, upper No. 2	–
	Berth, lower No. 2	–
	Berth, lower No. 4	+
B. subtilis	Toilet room	+
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 2	+
	Berth, lower No. 4	+
B. tuberculosis	Floor main sleeping compartment	+
	Sputum exposed by the Wilson method and injected into guinea pig; pig chloroformed 92d day; increased 415 grams in weight; spleen had only slight appearance of tuberculosis.	
	Floor main sleeping compartment	+
	Sputum exposed on carpet and injected into guinea pig; pig died of tuberculosis on 90th day.	
	Control guinea pig died of tuberculosis 63d day.	

EXPERIMENT NO. 49.

Formalin 2,000 c. c., permanganate 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.

Weather: Clear, light southerly wind.

Temperature in car at beginning of experiment, 75.5° F.

Relative humidity in car at beginning of experiment, 56 per cent.

Time of exposure, 2 hours (1.10 p. m. to 3.10 p. m.).

Temperature in car after opening and airing 15 minutes, 77.5° F.

Relative humidity in car after opening and airing 15 minutes, 47 per cent.

[+ means growth; – no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	–
	Drawing-room	–
	Smoking room	+
	Berth, upper No. 8	–
	Berth, lower No. 4	+
B. diphtheriæ	Toilet room	–
	Drawing-room	–
	Smoking room	+
	Berth, upper No. 9	+
	Berth, lower No. 4	–
B. typhosus	Toilet room	–
	Smoking room	–
	Drawing-room	–
	Berth, upper No. 9	–
	Berth, lower No. 4	+
B. subtilis	Toilet room	+
	Smoking room	+
	Drawing-room	+
	Floor, main sleeping compartment	+
	Berth, upper No. 10	+

EXPERIMENT NO. 50.

Formalin 2,000 c. c., permanganate 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.
Weather: Clear, rather strong southerly wind.
Temperature in car at beginning of experiment, 78° F.
Relative humidity in car at beginning of experiment, 53 per cent.
Time of exposure, 2 hours (12.30 p. m. to 2.30 p. m.).
Temperature in car after opening and airing 15 minutes, 79° F.
Relative humidity in car after opening and airing 15 minutes, 50 per cent.

[+ means growth; — no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	—
	Smoking room	+
	Drawing-room	+
	Berth, upper No. 9	+
	Berth, lower No. 6	+
B. pyocyaneus	Toilet room	—
	Drawing-room	+
	Smoking room	+
	Floor, main sleeping compartment	+
	Berth, upper No. 1	—
	Berth, upper No. 4	+
	Berth, upper No. 9	+
	Berth, lower No. 7	+
	Berth, lower No. 12	+
B. typhosus	Toilet room	—
	do	—
	Smoking room	—
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 5	—
	Berth, upper No. 6	+
	Berth, lower No. 1	—
	Berth, lower No 11	—
Staph. pyog. aur	Toilet room	—
	Smoking room	+
	Drawing-room	+
	Berth, upper No. 9	+
	Berth, lower No. 6	+
B. tuberculosis	Berth, upper No. 1	+
	Sputum exposed and injected into guinea pig; pig chloroformed 75th day; lost 25 grams in weight; marked lesions of tuberculosis.	
	Berth, lower No. 12	+
	Sputum exposed and injected into guinea pig; pig chloroformed 75th day; lost 30 grams in weight; marked lesions of tuberculosis.	
	No control pig inoculated.	

EXPERIMENT NO. 51.

Formalin 2,000 c. c., permanganate 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.
Weather: Clear moderate westerly wind.
Temperature in car at beginning of experiment, 71° F.
Relative humidity in car at beginning of experiment, 48 per cent.
Time of exposure, 2 hours (12 m. to 2 p. m.).
Temperature in car after opening and airing 15 minutes, 74° F.
Relative humidity in car after opening and airing 15 minutes, 58 per cent.

[+ means growth; — no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	+
	Smoking room	+
	Drawing room	+
	Berth, upper No. 2	+
	Berth, lower No. 7	+
B. pyocyaneus	Toilet room	+
	do	+
	Smoking room	+
	Floor main sleeping compartment	+
	Berth, upper No. 2	+
	Berth, upper No. 11	+
	Berth, lower No. 5	+
B. typhosus	Toilet room	—
	do	—
	Smoking room	—
	Drawing room	+
	Floor main sleeping compartment	+
	Berth, upper No. 11	—
	Berth, lower No. 12	+
Staph. pyog. aur	Toilet room	+
	Smoking room	+
	Floor main sleeping compartment	+
	Berth, upper No. 1	+
	Berth, lower No. 6	+
B. tuberculosis	Floor main sleeping compartment	+
	Sputum exposed and injected into guinea pig; pig chloroformed 77th day; increased 50 grams in weight; marked lesions of tuberculosis.	
	Berth, upper No. 10	+
	Sputum exposed and injected into guinea pig; pig chloroformed 77th day; increased 80 grams in weight; marked lesions of tuberculosis.	
	No control pig inoculated.	

EXPERIMENT NO. 52.

Formalin 2,000 c. c., permanganate 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.

Weather: Clear, rather strong northerly wind.

Temperature in car at beginning of experiment, 70° F.

Relative humidity in car at beginning of experiment, 48 per cent.

Time of exposure, 2 hours (11.25 a. m. to 1.25 p. m.).

Temperature in car after opening and airing 15 minutes, 72° F.

Relative humidity in car after opening and airing 15 minutes, 45 per cent.

[+ means growth; — no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	—
	Smoking room	—
	Floor main sleeping compartment	+
	Berth, upper No. 10	—
	Berth, lower No. 9	+
B. diphtheriæ	Toilet room	—
	Smoking room	+
	Drawing room	+
	Floor main sleeping compartment	+
	Berth, upper No. 8	—
B. typhosus	Toilet room	—
	Smoking room	—
	Floor main sleeping compartment	+
	Berth, upper No. 4	—
	Berth, lower No. 10	+
B. subtilis	Smoking room	+
	Drawing room	+
	Floor main sleeping compartment	+
	Berth, upper No. 2	+
	Berth, lower No. 6	+
B. tuberculosis	Floor of main sleeping compartment Sputum exposed and injected into guinea pig; pig chloroformed 87th day; increased 125 grams in weight; tuberculous lesions in liver, spleen, lymph glands, etc.	+
	Floor of main sleeping compartment Sputum exposed on carpet and injected into guinea pig; pig chloroformed 87th day; increased 40 grams in weight; marked tuberculous lesions in liver, spleen, glands, etc. Control guinea pig died of tuberculosis 50th day.	+

EXPERIMENT NO. 53.

Formalin 2,000 c. c., permanganate 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.

Weather: Clear, practically no wind.

Temperature in car at beginning of experiment, 65° F.

Relative humidity in car at beginning of experiment, 44 per cent.

Time of exposure, 2 hours (8.25 a. m. to 10.25 a. m.).

Temperature in car after opening and airing 15 minutes, 69° F.

Relative humidity in car after opening and airing 15 minutes, 55 per cent.

[+ means growth; − no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Smoking room	+
	Drawing room	−
	Floor main sleeping compartment	−
	Berth, lower No. 8	+
	Berth, lower No. 4	−
B. diphtheriæ	Drawing room	+
	Smoking room	+
	Floor main sleeping compartment	+
	Berth, upper No. 6	+
	Berth, lower No. 10	+
B. typhosus	Drawing room	+
	Smoking room	+
	Floor main sleeping compartment	+
	Berth, upper No. 10	−
	Berth, lower No. 10	+
B. subtilis	Toilet room	+
	Smoking room	+
	Floor main sleeping compartment	+
	Berth, upper No. 7	+
	Berth, lower No. 4	+
B. tuberculosis	Floor of main sleeping compartment	+
	Sputum exposed and injected into guinea pig; pig chloroformed 86th day; increased 35 grams in weight; tuberculous lesions in liver, spleen.	
	Floor of main sleeping compartment	+
	Sputum exposed on carpet and injected into guinea pig; pig chloroformed 86th day; increased 70 grams in weight; tuberculous lesions in spleen, mesentery, lymph glands.	
	Control guinea pig died of tuberculosis 50th day.	

The above experiment was done in the morning (8.25 to 10.25), at which time the relative humidity is high; but as the car had been artificially heated the night before the humidity was low. This probably accounts for the inefficient results obtained.

In the following experiment, temperature 46° F., polymerization was so marked that the deposit of paraform gave the interior furnishings of the car a frosty or white appearance.

EXPERIMENT NO. 54.

Formalin, 2,000 c. c., permanganate, 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.

Weather: Very cloudy, some melting snow on ground, no wind.

Temperature in car at beginning of experiment, 46° F.

Relative humidity in car at beginning of experiment, 45 per cent.

Time of exposure, 2 hours (11.25 a. m. to 1.25 p. m.).

Temperature in car after opening and airing 15 minutes, 47° F.

Relative humidity in car after opening and airing 15 minutes, 46 per cent.

[+ means growth; – no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	+
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 4	+
	Berth, lower No. 8	+
B. diphtheriæ	Floor main sleeping compartment	+
	Berth, upper No. 10	+
	Berth, lower No. 6	+
B. typhosus	Toilet room	+
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 6	+
	Berth, lower No. 4	+
B. subtilis	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 6	+

As a rule, in this climate the relative humidity is higher at night than during the day, the reasons for which are obvious. The following three experiments were therefore done at nighttime of particularly dry days, and, as will be seen, gave better germicidal results than would have been obtained during the daytime.

To show this difference in humidity, the official relative humidity at 2 p. m. of the day on which these experiments were done is therefore given in each experiment.

This matter is of practical importance in comparatively dry climates, and shows the advantage of doing the work at nighttime. Of course still better results would be obtained by continuing the experiments until the next morning. However, the same results would not be obtained by starting the experiments during the day with a low humidity and continuing during the night, as much of the formaldehyde would be lost before the humidity was raised; and, furthermore, the car being closed, practically the only change in humidity in the car would be as a result of the fall in temperature during the night.

EXPERIMENT NO. 55.

Formalin, 2,000 c. c., permanganate, 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in main sleeping compartment and one in the alleyway at each end.

Weather: Clear, moon shining, very little breeze.

Temperature in car at beginning of experiment, 77° F.

Relative humidity in car at beginning of experiment, 69 per cent.

Time of exposure, 2 hours (8 p. m. to 10 p. m.).

Temperature in car after opening and airing 15 minutes, 72° F.

Relative humidity in car after opening and airing 15 minutes, 71 per cent.

Official relative humidity at 2 p. m. of the date on which the experiment was done, 26 per cent.

[+ means growth; — no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Drawing-room	—
	Smoking room	—
	Floor main sleeping compartment	—
	Berth, upper No. 7	—
	Berth, lower No. 6	—
B. diphtheriæ	Toilet room	—
	Smoking room	—
	Floor main sleeping compartment	—
	Berth, lower No. 10	—
B. typhosus	Toilet room	—
	Smoking room	—
	Floor main sleeping compartment	—
	Berth, lower No. 8	—
	Berth, upper No. 11	—
B. subtilis	Toilet room	—
	Smoking room	—
	Floor main sleeping compartment	+
	Berth, upper No. 8	+
	Berth, lower No. 9	+
B. tuberculosis	Floor main sleeping compartment	+
	Sputum exposed by Wilson method and injected into guinea pig; pig chloroformed 88th day; increased 115 grams in weight; lesions of tuberculosis in spleen, liver, lungs, and lymph glands.	
	Floor main sleeping compartment	+
	Sputum exposed on carpet and injected into guinea pig; pig chloroformed 88th day; increased but 5 grams in weight; lesions of tuberculosis in liver and spleen.	
	Control guinea pig died of tuberculosis 63d day.	

EXPERIMENT NO. 56.

Formalin 2,000 c. c., permanganate 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in main sleeping compartment and one in the alleyway at each end.

Weather: Clear, moon shining, no wind.

Temperature in car at beginning of experiment, 61.5° F.

Relative humidity in car at beginning of experiment, 64 per cent.

Time of exposure, 2 hours (8.35 p. m. to 10.35 p. m.).

Temperature in car after opening and airing 15 minutes, 57° F.

Relative humidity in car after opening and airing 15 minutes, 66 per cent.

Official relative humidity at 2 p. m. of the date on which the experiment was done, 39 per cent.

[+ means growth; – no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	–
	Smoking room	–
	Floor main sleeping compartment	–
	Berth, upper No. 4	–
	Berth, lower No. 9	+
B. diphtheriæ	Drawing-room	–
	Smoking room	–
	Floor main sleeping compartment	–
	Berth, lower No. 10	–
	Berth, upper No. 8	–
B. typhosus	Toilet room	–
	Smoking room	–
	Drawing-room	
	Floor main sleeping compartment	–
	Berth, lower No. 6	–
B. subtilis	Drawing-room	+
	Smoking room	–
	Floor main sleeping compartment	–
	Berth, upper No. 5	+
	Berth, lower No. 4	+
B. tuberculosis	Floor main sleeping compartment	–
	Sputum exposed by Wilson method and injected into guinea pig; pig chloroformed 63d day; increased 270 grams in weight; no lesions of tuberculosis.	
	Floor main sleeping compartment	+
	Sputum exposed on carpet and injected into guinea pig; pig chloroformed 63d day; increased 150 grams in weight; lesions of tuberculosis in liver, spleen, and lymph glands. Control guinea pig died of tuberculosis 37th day.	

EXPERIMENT NO. 57.

Formalin 1,500 c. c., permanganate 750 grams, equally distributed in three iron buckets, one of which was placed in the main sleeping compartment of car and one in the alleyway at each end.
Weather: Clear and moonshiny, no wind.
Temperature in car at beginning of experiment, 59° F.
Relative humidity in car at beginning of experiment, 57 per cent.
Time of exposure, 2 hours (8.40 p. m. to 10.40 p. m.).
Temperature in car after opening and airing 20 minutes, 55° F.
Relative humidity in car after opening and airing 20 minutes, 62 per cent.
Official relative humidity at 2 p. m. of the date on which the experiment was performed, 39 per cent.

[+ means growth; – no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	–
	Smoking room	–
	Floor main sleeping compartment	+
	Berth, lower No. 6	+
B. diphtheriæ	Toilet room	–
	Smoking room	+
	Floor main sleeping compartment	–
	Berth, upper No. 5	–
	Berth, lower No. 10	–
B. typhosus	Smoking room	–
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 10	–
	Berth, lower No. 4	+
B. subtilis	Toilet room	+
	Smoking room	+
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 4	+
B. tuberculosis	Floor main sleeping compartment	–
	Sputum exposed by the Wilson method and injected into guinea pig; pig chloroformed 63d day; increased 370 grams in weight; no lesions of tuberculosis.	
	Floor main sleeping compartment	+
	Sputum exposed on carpet and injected into guinea pig; pig chloroformed 63d day; increased 160 grams in weight; spleen, lungs, and lymph glands tuberculous. Control guinea pig died of tuberculosis 37th day.	

II. DILUTED FORMALIN-PERMANGANATE METHOD.

The following four experiments were done with smaller quantities of formalin diluted with varying quantities of water before mixing with the permanganate. All except the first were done on comparatively dry days, with the object of determining whether the addition of the water would sufficiently increase the humidity to obtain efficient results on such days, as it has been satisfactorily demonstrated in previous experiments that moisture is one of the most essential factors in formaldehyde disinfection:

EXPERIMENT NO. 58.

Formalin 1,000 c. c., water 1,000 c. c., permanganate 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.

Weather: Clear, light southerly wind.

Temperature in car at beginning of experiment, 85° F.

Relative humidity in car at beginning of experiment, 73 per cent.

Time of exposure, 2 hours (11.30 a. m. to 1.30 p. m.).

Temperature in car after opening and airing 15 minutes, 88° F.

Relative humidity in car after opening and airing 15 minutes, 54 per cent.

[+ means growth, − no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	−
	Smoking room	−
	Drawing-room	−
	Floor main sleeping compartment	−
	Berth, lower No. 6	−
B. diphtheriæ	Smoking room	−
	Drawing-room	−
	Floor main sleeping compartment	−
	Berth, upper No. 6	−
	Berth, lower No. 8	−
B. typhosus	Toilet room	−
	Smoking room	−
	Drawing-room	−
	Floor main sleeping compartment	−
	Berth, upper No. 10	−
B. subtilis	Smoking room	+
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 6	+
B. tuberculosis	Smoking room	+
	Sputum exposed and injected into guinea pig; pig chloroformed 101st day; increased 200 grams in weight; marked lesions of tuberculosis in spleen and lymph glands, slight appearances in liver.	
	Floor main sleeping compartment	−
	Sputum exposed and injected into guinea pig; pig chloroformed 101st day; increased 240 grams in weight; no lesions of tuberculosis.	
	Control pig died of tuberculosis on 45th day.	

EXPERIMENT NO. 59.

Formalin 1,500 c. c., water 500 c. c., permanganate 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.

Weather: Dry and clear, light southwesterly wind.

Temperature in car at beginning of experiment, 76° F.

Relative humidity in car at beginning of experiment, 51 per cent.

Time of exposure, 2 hours (11.45 a. m. to 1.45 p. m.).

Temperature in car after opening and airing 15 minutes, 78° F.

Relative humidity in car after opening and airing 15 minutes, 36 per cent.

[+ means growth, − no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	−
	Drawing-room	−
	Floor main sleeping compartment	−
	Berth, lower No. 8	+
	Berth, upper No. 5	−
B. diphtheriæ	Toilet room	−
	Smoking room	+
	Floor main sleeping compartment	+
	Berth, upper No. 9	+
	Berth, lower No. 12	+
B. typhosus	Toilet room	−
	Drawing-room	−
	Floor main sleeping compartment	−
	Berth, upper No. 9	+
	Berth, lower No. 4	−
B. subtilis	Toilet room	−
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, lower No. 2	+
B. tuberculosis	Drawing-room	−
	Sputum exposed and injected into guinea pig; pig chloroformed 100th day; increased 270 grams in weight; no lesions of tuberculosis.	
	Floor main sleeping compartment	+
	Sputum exposed and injected into guinea pig; pig died of general tuberculosis on 88th day.	
	Control pig died of tuberculosis on 63d day.	

EXPERIMENT NO. 60.

Formalin 1,500 c. c., water 500 c. c., permanganate 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.
Weather: Clear and dry with light southwesterly winds.
Temperature in car at beginning of experiment, 64° F.
Relative humidity in car at beginning of experiment, 47 per cent.
Time of exposure, 2 hours (11.25 a. m. to 1.25 p. m.).
Temperature in car after opening and airing 15 minutes, 69° F.
Relative humidity in car after opening and airing 15 minutes, 43 per cent.

[+ means growth, – no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	–
	Smoking room	+
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 4	+
B. diphtheriæ	Toilet room	–
	Smoking room	+
	Floor main sleeping compartment	+
	Berth, upper No. 2	+
	Berth, lower No. 7	+
B. typhosus	Smoking room	–
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 6	+
	Berth, upper No. 10	+
B. subtilis	Smoking room	+
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 8	....
	Berth, lower No. 6	....
B. tuberculosis	Smoking room	–
	Sputum exposed and injected into guinea pig; pig chloroformed 90th day; increased 300 grams in weight; no lesions of tuberculosis.	
	Floor main sleeping compartment	–
	Sputum exposed and injected into guinea pig; pig chloroformed 90th day; increased 300 grams in weight; no lesions of tuberculosis.	
	Control pig died of tuberculosis on 63d day.	

EXPERIMENT NO. 61.

Formalin, 1,500 c. c.; water, 500 c. c.; permanganate, 1,000 grams, equally distributed in four galvanized-iron buckets, two of which were placed in the main sleeping compartment of car and one in the alleyway at each end.

Weather: Clear, southerly wind.

Temperature in car at beginning of experiment, 79° F.

Relative humidity in car at beginning of experiment, 66 per cent.

Time of exposure, 2 hours (12 m. to 2 p. m.).

Temperature in car after opening and airing 15 minutes, 86° F.

Relative humidity in car after opening and airing 15 minutes, 53 per cent.

[+ means growth; −, no growth.]

Organism.	Place of exposure in car.	Result
B. coli communis	Toilet room	−
	Smoking room	−
	Drawing-room	−
	Floor main sleeping compartment	−
	Berth, lower No. 11	−
B. diphtheriæ	Toilet room	−
	Smoking room	+
	Drawing-room	+
	Floor main sleeping compartment	−
	Berth, upper No. 8	+
B. typhosus	Toilet room	−
	Smoking room	−
	Floor main sleeping compartment	−
	Berth, upper No. 10	−
	Berth, lower No. 6	−
B. subtilis	Smoking room	−
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 4	−
B. tuberculosis	Smoking room	+
	Sputum exposed and injected into guinea pig; pig chloroformed 100th day; increased 200 grams in weight; lesions of tuberculosis in spleen and lymph glands.	
	Floor main sleeping compartment	+
	Sputum exposed and injected into guinea pig; pig chloroformed 100th day; marked lesions of tuberculosis in liver and spleen.	
	Control pig died of tuberculosis on 60th day.	

The results of the previous experiments done on dry days show that the organisms were usually not destroyed, and consequently no practical advantage gained by adding the water to the formalin. However, experiment No. 58 again proves our conclusions, namely, that successful disinfection does not depend so much upon the amount of gas as upon the temperature and humidity. Although a less quantity of formalin was used in this experiment than in the other three, the results were very much better.

III. RETORT WITHOUT PRESSURE.

A few experiments were done with this method, and as only one retort was used in each experiment, it was changed from one end of the car to the other at definite intervals during the process of vaporizing the formalin.

The locks of the car doors were removed in order to introduce the nozzle of the retort. The heat was under the retort 30 to 40 minutes before gas began to be given off, and then from 1 hour 30 minutes to 2 hours, depending upon temperature conditions in the different experiments, was required to completely vaporize the formalin. In one experiment, temperature 52° F., about 300 c. c. of liquid still remained in the retort at the end of the experiment. The time of exposure of the organisms was counted from the time of beginning to introduce the gas.

The experiments are as follows:

EXPERIMENT NO. 62.

Retort without pressure; formalin 2,000 c. c.
Time required to vaporize formalin, 1 hour 40 minutes.
Retort changed from one end of the car to the other every 20 minutes during process of vaporizing the formalin.
Weather: Damp and cloudy, light wind.
Temperature in car at beginning of experiment, 78° F.
Relative humidity in car at beginning of experiment, 71 per cent.
Time of exposure, 2 hours, from time of beginning to introduce the gas (11 a. m. to 1.00 p. m.)
Temperature in car after opening and airing 30 minutes, 81° F.
Relative humidity in car after opening and airing 30 minutes, 65 per cent.

[+ means growth; –, no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Smoking room	–
	Drawing-room	–
	Floor main sleeping compartment	–
	Berth, upper No. 8	–
	Berth, lower No. 6	–
B. diphtheriæ	Smoking room	–
	Drawing-room	–
	Floor main sleeping compartment	–
	Berth, upper No. 4	–
	Berth, lower No. 10	–
B. typhosus	Smoking room	–
	Drawing-room	–
	Floor main sleeping compartment	–
	Berth, upper No. 6	–
	Berth, lower No. 4	–
B. subtilis	Toilet room	–
	Smoking room	–
	Floor main sleeping compartment	+
	Berth, upper No. 2	+
	Berth, lower No. 8	+
B. tuberculosis	Floor main sleeping compartment	+
	Sputum exposed and injected into guinea pig; pig chloroformed 90th day; increased 190 grams in weight; tuberculous lesions in spleen and lymph glands.	
	Floor main sleeping compartment	+
	Sputum exposed on carpet and injected into guinea pig; pig died of tuberculosis on 50th day.	
	Control guinea pig died of tuberculosis on 60th day.	

EXPERIMENT NO. 63.

Retort without pressure; formalin 2,000 c. c.
Time required to vaporize formalin 1 hour and 30 minutes.
Retort changed from one end of car to the other every 15 minutes during process of vaporizing the formalin.
Weather: Dry and clear, light southwesterly wind.
Temperature in car at beginning of experiment, 73° F.
Relative humidity in car at beginning of experiment, 57 per cent.
Time of exposure, 2 hours, from time of beginning to introduce the gas (12 m. to 2 p. m.).
Temperature in car after opening and airing 15 minutes, 78° F.
Relative humidity in car after opening and airing 15 minutes, 38 per cent.

[+means growth; —, no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	—
	Drawing room	—
	Floor, main sleeping compartment	+
	Berth, upper No. 4	+
	Berth, lower No. 8	+
B. diphtheriæ	Drawing room	+
	Floor, main sleeping compartment	+
	Berth, upper No. 8	+
	Berth, upper No. 10	—
	Berth, lower No. 12	+
B. typhosus	Toilet room	—
	Drawing room	—
	Floor, main sleeping compartment	+
	Berth, lower No. 7	—
B. subtilis	Toilet room	—
	Drawing room	+
	Floor, main sleeping compartment	+
	Berth, upper No. 12	+
	Berth, lower No. 8	+
B. tuberculosis	Drawing room Sputum exposed and injected into guinea pig; pig chloroformed 97th day; marked lesions of tuberculosis in spleen.	+
	Floor, main sleeping compartment Sputum exposed and injected into guinea pig; pig chloroformed 97th day; increased 300 grams in weight; no lesions of tuberculosis. Control guinea pig died of tuberculosis 63d day.	—

EXPERIMENT No. 64.

Retort without pressure; formalin 2,000 c. c.

The process of vaporizing the formalin was continued for 2 hours, at the end of which time about 300 c. c. of fluid remained in the retort. During this time the retort was changed from one end of car to the other every 15 minutes.

Weather: Cloudy, melting snow, no wind.

Temperature in car at beginning of experiment, 52° F.

Relative humidity in car at beginning of experiment, 40 per cent.

Time of exposure, 2 hours, from time of beginning to introduce the gas (12 m. to 2 p. m.).

Temperature in car after opening and airing 15 minutes, 52° F.

Relative humidity in car after opening and airing 15 minutes, 40 per cent.

[+ means growth; −, no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	+
	Drawing room	+
	Floor, main sleeping compartment	+
	Berth, upper No. 4	+
	Berth, lower No. 8	+
B. diphtheriæ	Floor, main sleeping compartment	+
	Berth, upper No. 10	+
	Berth, lower No. 6	+
B. typhosus	Toilet room	−
	Drawing room	+
	Floor, main sleeping compartment	+
	Berth, upper No. 6	+
	Berth, lower No. 4	+
B. subtilis	Drawing room	+
	Floor, main sleeping compartment	+
	Berth, upper No. 6	+

With this method all the guinea pigs showed lesions of tuberculosis, one in experiment No 62 dying before the control. The others only showed a diminished virulence of the organism. Otherwise the results obtained in the above experiments agree rather closely with those in the formalin-permanganate method under similar conditions of temperature and humidity.

As much of the 2 hours is consumed in introducing the formaldehyde gas, doubtless more advantage would be gained by a longer exposure with this method than would be the case with the formalin-permanganate method. Although the temperature was 73° F., and humidity 57 per cent in experiment No. 63, the latter was not high enough to give efficient results. This indicates that about the same percentage of humidity is required in this method as in the formalin-permanganate method.

IV. AUTOCLAVE UNDER PRESSURE.

About 20 minutes were required to raise the pressure in the autoclave to 60 pounds. The pressure was kept between 40 and 60 pounds while vaporizing the formalin. This process required from 40 to 60 minutes in the different experiments, after which practically no liquid remained in the autoclave.

During this process the autoclave was changed from one end of the car to the other every 10 or 15 minutes. As with the retort, it was necessary to remove the locks from the doors in order to introduce the nozzle of the autoclave.

The experiments are as follows:

EXPERIMENT NO. 65.

Autoclave, formalin 2 000 c. c.
Time required to vaporize formalin, 45 minutes.
Autoclave changed from one end of car to other every 15 minutes during process of vaporizing the formalin.
Weather: Clear; southerly breeze.
Temperature in car at beginning of experiment, 78° F.
Relative humidity at beginning of experiment, 67 per cent.
Time of exposure, 2 hours (11.55 a. m. to 1.55 p. m.) from time of beginning to introduce the gas.
Temperature in car after opening and airing 30 minutes, 87° F.
Relative humidity after opening and airing 30 minutes, 50 per cent.

[+ means growth; –, no growth].

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	–
	Smoking room	–
	Drawing-room	–
	Floor main sleeping compartment	–
	Berth, upper No. 8	–
B. diphtheriæ	Toilet room	–
	Smoking room	–
	Floor main sleeping compartment	+
	Berth, upper No. 8	–
	Berth, lower No. 4	+
B. typhosus	Toilet room	–
	Smoking room	–
	Drawing-room	–
	Floor main sleeping compartment	–
	Berth, lower No. 10	–
B. subtilis	Smoking room	–
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 6	–
B. tuberculosus	Smoking room	–
	Sputum exposed and injected into guinea pig. Pig chloroformed 100th day—increased 270 grams in weight; no lesion of tuberculosis.	+
	Floor main sleeping compartment.	
	Sputum exposed and injected into guinea pig. Pig chloroformed 100th day—increased 140 grams in weight; only the spleen showed lesions of tuberculosis.	
	Control guinea pig died of tuberculosis 60th day.	

EXPERIMENT NO. 66.

Autoclave, formalin 2,000 c. c.
Time required to vaporize formalin, 40 minutes.
Autoclave changed from one end of car to other every 10 minutes during the process of vaporizing the formalin.
Weather: Clear; very little wind.
Temperature in car at beginning of experiment, 74.5° F.
Relative humidity at beginning of experiment, 64 per cent.
Time of exposure, 2 hours (11.35 a. m. to 1.35 p. m.) from time of beginning to introduce the gas.
Temperature in car after opening and airing 25 minutes, 83° F.
Relative humidity after opening and airing 25 minutes, 40 per cent.

[+ means growth; –, no growth].

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	–
	Smoking room	–
	Drawing-room	–
	Floor main sleeping compartment	–
	Berth, lower No. 8	–
B. diphtheriæ	Smoking room	–
	Drawing-room	–
	Floor main sleeping compartment	+
	Berth, upper No. 9	+
	Berth, lower No. 6	+
B. typhosus	Toilet room	–
	Smoking room	–
	Drawing-room	–
	Floor main sleeping compartment	–
	Berth, lower No. 10	–
B. subtilis	Toilet room	–
	Smoking room	+
	Floor main sleeping compartment	+
	Berth, upper No. 7	+
	Berth, lower No. 3	+
B. tuberculosus	Floor main sleeping compartment Sputum exposed by Wilson method and injected into guinea pig. Pig chloroformed 95th day—increased 410 grams in weight; no lesions of tuberculosis.	–
	Floor main sleeping compartment Sputum exposed on carpet and injected into guinea pig. Pig died of tuberculosis 59th day. Control guinea pig died of tuberculosis 60th day.	+

EXPERIMENT NO. 67.

Autoclave, formalin 2,000 c. c.

Time required to vaporize formalin, 50 minutes.

Autoclave changed from one end of car to other every 10 minutes during the process of vaporizing the formalin.

Weather: Cloudy; moderate northeast wind.

Temperature in car at beginning of experiment, 47° F.

Relative humidity at beginning of experiment, 53 per cent.

Time of exposure, 2 hours (12.25 p. m. to 2.25 p. m.) from time of beginning to introduce the gas

Temperature in car after opening and airing 15 minutes, 40° F.

Relative humidity after opening and airing 15 minutes, 52 per cent.

[+ means growth; –, no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	+
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 12	+
	Berth, lower No. 8	+
B. diphtheriæ	Toilet room	+
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 11	+
B. typhosus	Toilet room	+
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 7	+
	Berth, lower No. 3	+
B. subtilis	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, upper No. 1	+
	Berth, lower No. 4	+

The above results do not differ materially from those with the other methods described above. For comparison, experiments Nos. 46 and 66 were done simultaneously, as were No. 61 and 65. Of the four guinea pigs used in this method two showed no lesions of tuberculosis, while the other two showed that the virulence of the organism had been diminished as compared with the controls.

A longer exposure would no doubt have given better results, especially in experiments Nos. 65 and 66. Evidently about the same temperature and humidity are required in this method to accomplish efficient disinfection as in the other methods.

V. GENERATING LAMP.

Two lamps were used in an experiment, placed one in either end of the main compartment of the sleeping car. Fifteen hundred c. c. of commercial wood alcohol were used in each lamp.

The lamps were started, as previously described (p. 57), in the room experiments. As soon as they began to give off formaldehyde gas, etc., the car was closed and the time of exposure of the organisms counted from that time. At the conclusion of the experiments the process of oxidation of the alcohol seemed to be completed except in experiment No. 71. In this experiment, temperature 50° F., both lamps were still burning at the end of 2 hours.

EXPERIMENT NO. 68.

Two generating lamps; commercial wood alcohol 3,000 c. c. (1,500 c. c. in each lamp).[a]
Lamps placed one in each end of main sleeping compartment of car.
Weather: Damp and cloudy, light wind.
Temperature in car at beginning of experiment, 77° F.
Relative humidity in car at beginning of experiment, 71 per cent.
Time of exposure, 2 hours (11.40 a. m. to 1.40 p. m.) from time of starting lamps and closing car.
Temperature in car after opening and airing 20 minutes, 81° F.
Relative humidity in car after opening and airing 20 minutes, 72 per cent.

[+ means growth, – no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	–
	Smoking room	–
	Floor, main sleeping compartment	–
	Berth, upper No. 5	–
	Berth, lower No. 8	–
B. diphtheriæ	Smoking room	–
	Drawing-room	–
	Floor, main sleeping compartment	+
	Berth, upper No. 4	–
	Berth, lower No. 4	–
B. typhosus	Toilet room	–
	Smoking room	–
	Drawing-room	–
	Floor, main sleeping compartment	–
	Berth, lower No. 9	–
B. subtilis	Smoking room	+
	Drawing-room	+
	Floor, main sleeping compartment	+
	Berth, upper No. 7	+
	Berth, lower No. 6	+
B. tuberculosis	Floor, main sleeping compartment	+
	Sputum exposed by Wilson method and injected into guinea pig; pig chloroformed 90th day; increased 230 grams in weight; tubercular lesions in liver, spleen.	
	Floor, main sleeping compartment	+
	Sputum exposed on carpet and injected into guinea pig; pig chloroformed 90th day; increased 100 grams in weight; marked lesions of tuberculosis in liver, spleen, lungs, diaphragm, and lymph glands.	
	Control guinea pig died of tuberculosis 60th day.	

---

[a] In experiments Nos. 68, 69, 70 and 71, the quantity of wood alcohol was about 10 per cent less than is required by U. S. Quarantine regulations.—[Ed.]

EXPERIMENT NO. 69.

Two generating lamps; commercial wood alcohol 3,000 c. c. (1,500 c. c. in each lamp).
Lamps placed one in each end of main sleeping compartment of car.
Weather: Clear and dry, very little wind.
Temperature in car at beginning of experiment, 60° F.
Relative humidity in car at beginning of experiment, 58 per cent.
Time of exposure, 2 hours (11.30 a. m. to 1.30 p. m.) from time of starting lamps and closing car.
Temperature in car after opening and airing 15 minutes, 62° F.
Relative humidity in car after opening and airing 15 minutes, 50 per cent.

[+ means growth, − no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	−
	Smoking room	+
	Floor, main sleeping compartment	+
	Berth, upper No. 4	+
	Berth, lower No. 10	−
B. diphtheriæ	Smoking room	+
	Drawing-room	+
	Floor, main sleeping compartment	+
	Berth, upper No. 6	+
	Berth, lower No. 5	+
B. typhosus	Toilet room	+
	Drawing-room	+
	Floor, main sleeping compartment	−
	Berth, upper No. 3	−
	Berth, lower No. 12	+
B. subtilis	Smoking room	+
	Drawing-room	+
	Floor, main sleeping compartment	+
	Berth, upper No. 10	+
	Berth, lower No. 6	+
B. tuberculosis	Floor, main sleeping compartment Sputum exposed by Wilson method and injected into guinea pig; pig chloroformed 63d day; increased 175 grams in weight; tubercular lesions in liver, spleen, and lymph glands.	+
	Floor, main sleeping compartment Sputum exposed on carpet and injected into guinea pig; pig died of tuberculosis on 50th day. Control guinea pig died of tuberculosis 37th day.	+

EXPERIMENT NO. 70.

Two generating lamps; commercial wood alcohol 3,000 c. c. (1,500 c. c. in each lamp).
Lamps placed one in each end of main sleeping compartment of car.
Weather: Clear and dry, light southwesterly breeze.
Temperature in car at beginning of experiment, 65° F.
Relative humidity in car at beginning of experiment, 48 per cent.
Time of exposure, 2 hours (11.15 a. m. to 1.15 p. m.) from time of starting lamps and closing car.
Temperature in car after opening and airing 10 minutes, 69° F.
Relative humidity in car after opening and airing 10 minutes, 39 per cent.

[+ means growth, — no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	+
	Smoking room	+
	Drawing-room	+
	Floor main sleeping compartment	—
	Berth, lower No. 6	+
B. diphtheriæ	Toilet room	+
	Smoking-room	+
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, lower No. 4	+
B. typhosus	Toilet room	—
	Smoking room	+
	Drawing-room	—
	Floor main sleeping compartment	—
	Berth, upper No. 3	—
B. subtilis	Smoking room	—
	Drawing-room	—
	Floor main sleeping compartment	—
	Berth, upper No. 7	—
	Berth, lower No. 8	—
B. tuberculosis	Smoking room	+
	Sputum exposed and injected into guinea pig; pig chloroformed 96th day; increased 300 grams in weight; tubercular lesions in spleen.	
	Floor main sleeping compartment	+
	Sputum exposed and injected into guinea pig; pig chloroformed 96th day; increased 230 grams in weight; liver, spleen, and lymph glands show lesions of tuberculosis.	
	Control guinea pig died of tuberculosis 63d day.	

---

[a] In experiments Nos. 68, 69, 70 and 71, the quantity of wood alcohol was about 10 per cent less than is required by U. S. Quarantine regulations.—[Ed.]

EXPERIMENT NO. 71.

Two generating lamps; commercial wood alchool 3,000 c. c. (1,500 c. c. in each lamp).
Lamps placed one in each end of main sleeping compartment of car.
Weather: Cloudy, moderate northeast wind.
Temperature of car at beginning of experiment, 50° F.
Relative humidity of car at beginning of experiment, 55 per cent.
Time of exposure, 2 hours (12.30 p. m. to 2.30 p. m.), from time of starting lamps and closing car.
Temperature in car after opening and airing 10 minutes, 45 ° F.
Relative humidity in car after opening and airing 10 minutes, 57 per cent.

[+ means growth, — no growth.]

Organism.	Place of exposure in car.	Result.
B. coli communis	Toilet room	+
	Smoking room	+
	Drawing-room	+
	Floor main sleeping compartment	+
	Berth, lower No. 10	+
B. diphtheriæ	Toilet room	+
	Smoking room	+
	Berth, upper No. 10	+
B. typhosus	Toilet room	+
	Smoking room	+
	Floor main sleeping compartment	+
	Berth, upper No. 2	+
	Berth, lower No. 6	+
B. subtilis	Toilet room	+
	Smoking room	+
	Floor main sleeping compartment	+
	Berth, upper No. 8	+

The results in experiment No. 68 are satisfactory except that the tubercle bacilli were not killed, although their virulence was diminished. In the other experiments most of the organisms escaped destruction, due no doubt to the low relative humidity and temperature.

In all the experiments with this method it was possible to enter the car almost as soon as it was opened. Consequently, no special advantage would likely have been gained by a longer exposure in the above experiments. Although this method increased the humidity considerably, it did not do so sufficiently in experiment No. 69 (humidity 58 per cent) to accomplish efficient results.

SUMMARY AND CONCLUSIONS.

Formaldehyde gas, regardless of the method by which it is evolved, is a powerful surface disinfectant under certain conditions. Successful disinfection with it is so dependent upon several factors that its usefulness is more or less limited. The temperature and humidity are of primary importance, while the influence of the winds, character of the room, etc., are of secondary importance.

The effects of temperature seem to be principally upon the state of the formaldehyde after it is liberated; that is, below a certain point it polymerizes. This seems to take place below about 65° F. with the formalin-permanganate and below about 60° F. with the other methods. Although the germicidal power of formaldehyde is not entirely lost at these temperatures, it is so diminished as to be of very little practical value for disinfection purposes.

The rôle played by the relative humidity in formaldehyde disinfection is more important than that of any other other influencing factors. In the absence of moisture, formaldehyde is practically inert as a germicide. The minimum percentage of relative humidity that invariably gives efficient results in disinfecting with formaldehyde can not be stated exactly, since it varies slightly according to other conditions, such as temperature, wind, the quantity of gas present, the space to be disinfected, etc.

However, it appears from experience that the relative humidity in the air before starting an experiment should not be lower than as follows: (1) For an ordinary well-closed room, 60 per cent; (2) for ordinary railway cars, 65 per cent. With a high temperature, a large quantity of formaldehyde, and a long exposure, efficient results may be obtained with slightly lower percentages of humidity than just stated.

There is not much difference in the amount of moisture given off by the different methods. This form of moisture, however, does not seem to answer for disinfection purposes so well as the natural humidity in the atmosphere. This conclusion is indicated in some experiments in which the relative humidity before starting the experiment was below the point above stated, and while the moisture given off raised the percentage of humidity to a point where efficient results would be expected, the organisms usually escaped destruction. Consequently, the exact value of the moisture given off in the process of liberating the formaldehyde can not be positively stated, but it appears that too much trust must not be placed in it.

In the experiments in a bottle very good results were obtained with a relative humidity between 50 and 60 per cent, which is slightly less than that required in the room and car experiments. Of course, the conditions in the bottle were much more favorable for efficient results than usually obtain in ordinary formaldehyde disinfection.

The results of our chemical determinations must be interpreted as representing the quantities of formaldehyde gas that may be expected to be present in a fairly air-tight room when charged with 600 c. c. of formalin by the various methods described and after a certain interval of time which varies with the method used.

According to the maximum quantity of gas found, on an average warm day and regardless of the time required to evolve it, the methods may be arranged as follows:

	Per cent.
Retort without pressure	47.0
Autoclave	41.5
Formalin-permanganate	39.15
Diluted formalin-permanganate	35.1
Sheet spraying	30.48
Formalin-aluminum sulphate-lime	14.0

The above figures simply represent the percentage of formaldehyde gas found, as compared with the quantity contained in the amount of formalin used in an experiment.

As alcohol is used in the generating lamp, it is impracticable at this time to compare the percentage of formaldehyde evolved by this method with that evolved by the above methods.

Under conditions as given above the methods may be arranged according to the maximum quantity of formaldehyde gas actually found per cubic foot of air space in the room, as follows:

	Grams.
Retort without pressure	0.05037
Autoclave	0.04448
Formalin-permanganate	0.0419
Diluted formalin-permanganate	0.03755
Sheet spraying	0.03262
Formalin-aluminum sulphate-lime	0.01498

Below a temperature of about 65° F. for the formalin-permanganate and about 60° F. for the other methods polymerization or other change in the formaldehyde takes place to such an extent that only a relatively small percentage of gas is obtained. This of course is more marked the lower the temperature.[a]

The different methods are affected in about the same proportion except in case of the sheet-spraying method. In this latter method it seems that the formaldehyde is not even given off when the temperature is below the point above stated. In the different experiments in the bottle with the formalin-permanganate method the percentages of formaldehyde liberated agree fairly well, but are slightly lower than in the room experiments under similar conditions of temperature. The difference is due in all probability to the small quantities of substances used in the former experiments.

The bottle experiments show that a low temperature does not materially affect the quantity of formaldehyde actually given off, and therefore the small percentages obtained in the room under this condition are principally due to the formaldehyde undergoing change after liberation.

---

[a] This polymerization, however, does not take place in the gas, as evolved by the formaldehyde generating lamp, the product of which does not seem to polymerize under any conditions of temperature at which experiments have, up to this time, been conducted.—[Ed.]

A small quantity of formaldehyde is efficient for surface disinfection, provided the temperature and humidity are high. A large quantity assists penetration, as also do high temperature and humidity; but even then the penetrating power of formaldehyde is so limited that it should not be employed for disinfection purposes requiring any marked degree of penetration.

Depending upon the conditions as stated, the quantity of formalin may vary from 200 to 500 c. c. per 1,000 cubic feet of air space. As sleeping cars have many ventilators, etc., the leakage of formaldehyde gas from them is quite rapid, so that it is necessary to use a relatively larger quantity of formalin than is usually required in room disinfection.

The length of exposure depends upon circumstances. Two hours under favorable conditions were usually sufficient in our experiments. In the car experiments very little formaldehyde gas was found in the car at the end of this time. Consequently very little would have been gained by a longer exposure. In a well-closed room a longer exposure would doubtless be of advantage, although our conclusions are that in surface disinfection with formaldehyde the organisms are mostly killed within the first hour of exposure if they are destroyed at all.

For simplicity and rapidity the formalin-permanganate method is far superior to any of the other methods tried. It liberates the formaldehyde gas almost instantaneously and in almost as large quantities as the retort and autoclave methods.

Better disinfection is accomplished by having a comparatively large quantity of formaldehyde gas and a short exposure than a small quantity and long exposure. The formalin-permanganate method is more applicable than any of the other methods for disinfecting an inclosure which is not comparatively tight. With this method a larger percentage of exposures of *B. tuberculosis* were killed than with any of the other methods.

The formalin and permanganate should be used in about the proportion of 1 c. c. of the former to 0.5 gram of the latter. No obvious advantage is gained by adding water to the formalin before mixing with the permanganate.

The retort, autoclave, and generating lamp are efficient methods under favorable conditions, but they require more or less complicated apparatus, as well as considerable time to evolve the formaldehyde gas.

The sheet-spraying method is rather simple and economical and is especially applicable for disinfection purposes requiring long exposure.

The formalin-aluminum sulphate-lime method is not so simple and efficient as some of the other methods, but under favorable conditions it may be used with very good results.

O

395